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(54) Title: METHODS AND COMPOSITIONS FOR MODULATING RESPONSIVENESS TO CORTICOSTEROIDS

(57) Abstract

Method for modulating responsiveness to corticosteroids in a subject are provided. In the method of the invention, an agent which antagonizes a target that regulates production of IFN- γ in the subject is administered to the subject in combination with a corticosteroid such that responsiveness of the subject to the corticosteroid is modulated as compared to when a corticosteroid alone is administered to the subject. In one embodiment, the agent is an IL-18 antagonist. In another embodiment, the agent is an interleukin-12 (IL-12) antagonist. In yet another embodiment, the agent is an NK cell antagonist. In a preferred embodiment, the agent is an inhibitor of a caspase family protease, preferably an ICE inhibitor. In another preferred embodiment, the agent is an anti-IL-12 monoclonal antibody. In yet another preferred embodiment, the agent is an anti-asialo-GM1 antibody or an NK1.1 antibody. Other preferred agents include phosphodiesterase IV inhibitors and beta-2 agonists. The methods of the invention can be used in the treatment of a variety of inflammatory and immunological diseases and disorders. Pharmaceutical compositions comprising an agent which antagonizes a target that regulates production of IFN- γ in a subject, a corticosteroid and a pharmaceutically acceptable carrier are also provided. A preferred composition comprises an ICE inhibitor, a corticosteroid and a pharmaceutically acceptable carrier.

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METHODS AND COMPOSITIONS FOR MODULATING RESPONSIVENESS TO CORTICOSTEROIDS

Background of the Invention

5 Standard therapy for a variety of immune and inflammatory disorders includes administration of corticosteroids, which have the ability to suppress immunologic and inflammatory responses. Corticosteroids are used in the treatment of disorders such as asthma, autoimmune diseases (*e.g.*, rheumatoid arthritis, systemic lupus erythematosus) and transplant rejection (for reviews on corticosteroids, see *e.g.*, Truhan, A.P. *et al.* (1989) *Annals of Allergy* 62:375-391; Baxter, J.D. (1992) *Hospital Practice* 27:111-134; Kimberly, R.P. (1992) *Curr. Opin. Rheumatol.* 4:325-331; Weisman, M.H. (1995) *Curr. Opin. Rheumatol.* 7:183-190). Corticosteroids are also used topically in the treatment of various dermatological disorders, such as contact dermatitis, psoriasis vulgaris, lichen planus, keloids and urticaria pigmentosa (for a review, see Sterry, W. (1992) *Arch. Dermatol. Res.* 284 (Suppl.):S27-S29).

 While therapeutically beneficial, the use of corticosteroids is associated with a number of side effects, ranging from mild to possibly life threatening. Complications associated with prolonged and/or high dose steroid usage include musculoskeletal effects (*e.g.*, osteoporosis, myopathy, aseptic necrosis of bone), ophthalmic effects (*e.g.*, posterior subcapsular cataracts), gastrointestinal effects (*e.g.*, ulcers, pancreatitis, nausea, vomiting), cardiovascular effects (*e.g.*, hypertension, atherosclerosis), central nervous system effects (*e.g.*, pseudotumor cerebri, psychiatric reactions), dermatological effects (*e.g.*, hirsutism, redistribution of subcutaneous fat, impaired wound healing, thinning of the skin) and suppression of the hypothalamus-pituitary-adrenal axis (see *e.g.*, Truhan, A.P. *et al.* (1989) *Annals of Allergy* 62:375-391). Many of the side effects of corticosteroid usage appear to be dose-dependent (Kimberly, R.P. (1992) *Curr. Opin. Rheumatol.* 4:325-331). Accordingly, methods and compositions that enable the use of a lower effective dosage of corticosteroids (referred to as the "steroid sparing effect") would be highly desirable to avoid unwanted side effects.

30 Another problem that limits the usefulness of corticosteroids is the phenomenon of steroid resistance. Certain inflammatory or immunological diseases exhibit refractoriness to steroid treatment. For example, attempts to use corticosteroid therapy to treat septic shock in humans have met with disappointing results and thus corticosteroids are not generally recommended as adjunctive therapy in severe sepsis or septic shock (see *e.g.*, Putterman, C. (1989) *Israel J. Med. Sci.* 25:332-338; Bone, R.C. and Brown, R.C. (1990) in Vincent, J.L. (ed.) *"Update in Intensive Care and Emergency Medicine 10"*, Heidelberg:Springer Verlag, p. 121). Other disorders that often exhibit resistance to corticosteroid treatment include inflammatory bowel disease (see *e.g.*, Hibi, T. *et al.* (1995)

J. Gastroenterol. 30:121-123) and graft-versus-host disease (Antin, J.H. *et al.* (1994) *Blood* 84:1342-1348; Racadot, E. *et al.* (1995) *Bone Marrow Transplantation* 15:669-677). Thus, methods and compositions that can be used to overcome or reverse corticosteroid resistance in inflammatory and immunological disorders are still needed.

Yet another disadvantage of corticosteroid therapy is the occurrence of a "steroid rebound effect" when corticosteroid administration is discontinued. A steroid rebound effect is characterized by the worsening of the inflammatory condition(s) being treated upon cessation of steroid therapy. Methods and compositions that can be used to ameliorate the steroid rebound effect are still needed.

Summary of the Invention

This invention provides methods and compositions for modulating responsiveness to corticosteroids in a subject. For example, the methods and compositions of the invention can be used to reverse steroid resistance in a subject, to thereby allow the subject to be treated with corticosteroids. The methods and compositions of the invention also can be used to increase steroid sensitivity in a subject, to thereby achieve therapeutic effectiveness of corticosteroid treatment at lower dosages (*e.g.*, to avoid harmful side effects of high doses of corticosteroids or to allow treatment of steroid-dependent diseases with lower doses). Still further, the methods and compositions of the invention can be used to ameliorate the steroid rebound effect when a subject undergoing corticosteroid treatment is taken off corticosteroids.

In the modulatory methods of the invention, an agent which antagonizes a target that regulates production of IFN- γ in a subject is administered to the subject in combination with a corticosteroid such that responsiveness of the subject to the corticosteroid is modulated as compared to when a corticosteroid alone is administered to the subject. The target which is antagonized can be, for example, a cytokine or enzyme that regulates IFN- γ production or a cell that regulates IFN- γ production. The agent is administered at a dosage and by a route sufficient to inhibit IFN- γ production in the subject. In various embodiments, the agent and the corticosteroid are administered at the same time, the agent is administered first and then the corticosteroid is administered or the corticosteroid is administered first and then the agent is administered. The methods can be applied to prophylactic and therapeutic regimens of corticosteroid treatment.

In one embodiment, the method involves administration of an agent that is an IL-18 antagonist. The IL-18 antagonist is administered at a dosage and by a route sufficient to inhibit IL-18 activity in the subject. The IL-18 antagonist can act, for example, at the level of IL-18 synthesis, IL-18 cytokine activity or IL-18 interaction with an IL-18 receptor. In a preferred embodiment, the IL-18 antagonist is an inhibitor of a caspase family protease, preferably an Interleukin-1 β Converting Enzyme (ICE) inhibitor. In another embodiment,

the IL-18 antagonist is an antibody, antibody fragment or engineered binding protein that binds to IL-18 or an IL-18 receptor.

In another embodiment, the method involves administration of an agent that is an Interleukin-12 (IL-12) antagonist. The IL-12 antagonist is administered at a dosage and by
5 a route sufficient to inhibit IL-12 activity in the subject. The IL-12 antagonist can act, for example, at the level of IL-12 synthesis, IL-12 cytokine activity or IL-12 interaction with an IL-12 receptor. In a preferred embodiment, the IL-12 antagonist is an antibody, antibody fragment or engineered binding protein that binds to IL-12 or an IL-12 receptor. In another preferred embodiment, the IL-12 antagonist is an agent that stimulates
10 production of cyclic AMP (cAMP) in cells that produce IL-12. Examples of agent that can be used to stimulate cAMP include phosphodiesterase IV inhibitors and beta-2 agonists. In yet another embodiment, the IL-12 antagonist is a STAT4 inhibitor.

In yet another embodiment, the method involves administration of an agent that depletes or eliminates NK cells and NK-like cells (referred to herein as an "NK cell
15 antagonist") from the subject. The NK cell antagonist is administered at a dosage and by a route sufficient to inhibit IFN- γ production in the subject. Preferred NK cell antagonists are antibodies specific for NK/NK-like cells that deplete these cells *in vivo*. Examples of preferred antibodies for use as NK cell antagonists are anti-asialo-GM1 antibodies and NK1.1 antibodies.

20 Another aspect of the invention pertains to a method for modulating responsiveness to corticosteroids in a subject, wherein an inhibitor of a caspase family protease, preferably ICE, is administered to the subject together with a corticosteroid, such that responsiveness of the subject to the corticosteroid is modulated as compared to when a corticosteroid alone is administered to the subject.

25 Yet another aspect of the invention pertains to a method for modulating responsiveness to corticosteroids in a subject, wherein an IL-12 antagonist is administered to the subject together with a corticosteroid, such that responsiveness of the subject to the corticosteroid is modulated as compared to when a corticosteroid alone is administered to the subject.

30 Yet another aspect of the invention pertains to a method for modulating responsiveness to corticosteroids in a subject, wherein an NK cell antagonist (e.g., an anti-NK/NK-like cell antibody) is administered to the subject together with a corticosteroid, such that responsiveness of the subject to the corticosteroid is modulated compared to when a corticosteroid alone is administered to the subject.

35 Still another aspect of the invention pertains to a method for modulating responsiveness to corticosteroids in a subject, wherein a subject in need of modulation of responsiveness to a corticosteroid is selected and an agent which antagonizes a target that regulates production of IFN- γ in the subject is administered to the subject such that

responsiveness of the subject to a corticosteroid is modulated as compared to when a corticosteroid alone is administered to the subject. The agent is administered to the subject at a dosage and by a route sufficient to inhibit IFN- γ production in the subject. The subject that is selected can be, for example, a subject that is steroid resistant prior to treatment, a
5 steroid-responsive subject in whom steroid sensitivity is to be increased or a subject to be taken off steroids in whom the steroid rebound effect is to be ameliorated.

The invention also provides pharmaceutical compositions for modulating responsiveness to corticosteroids in a subject. In one embodiment, a composition of the invention comprises an agent which antagonizes a target that regulates production of IFN- γ
10 in the subject, a corticosteroid and a pharmaceutically acceptable carrier. In another embodiment, a composition of the invention comprises an IL-18 antagonist (such as inhibitor of a caspase family protease, preferably an ICE inhibitor, or an anti-IL-18 or anti-IL-18 receptor monoclonal antibody), a corticosteroid and a pharmaceutically acceptable carrier. In yet another embodiment, a composition of the invention comprises an IL-12
15 antagonist (e.g., an anti-IL-12 or anti-IL-12 receptor monoclonal antibody, a phosphodiesterase IV inhibitor, a beta-2 agonist, a STAT4 inhibitor), a corticosteroid and a pharmaceutically acceptable carrier. In still another embodiment, a composition of the invention comprises an NK-cell antagonist (e.g., an anti-NK/NK-like cell antibody), a corticosteroid and a pharmaceutically acceptable carrier. The pharmaceutical compositions
20 of the invention can be formulated for administration via a preferred route of administration for achieving a desired therapeutic effect. In one preferred embodiment, the pharmaceutical composition is formulated for topical administration. In another preferred embodiment, the pharmaceutical composition is formulated for administration by inhalation. Other preferred routes of administration include oral and intravenous administration.

25 The methods and compositions of the invention can be used in the treatment of any disease or disorder in which it is desirable to modulate steroid responsiveness. In a preferred embodiments, the methods and compositions of the invention are used to treat a subject suffering from septic shock. In another embodiment, the methods and compositions of the invention are used to treat a subject suffering from Crohn's disease. In another
30 embodiment, the methods and compositions are used to treat a subject suffering from asthma. In another embodiment, the methods and compositions are used to treat a subject suffering from an autoimmune disease or disorder. In another embodiment, the methods and compositions are used to treat a subject suffering from graft-versus-host disease or transplant rejection. In yet another embodiment, the methods and compositions are used to
35 treat a subject suffering from an acute inflammatory disorder. In still another embodiment, the methods and compositions are used to treat a subject suffering from a chronic inflammatory disorder.

Brief Description of the Drawings

Figure 1 is a bar graph showing serum TNF α levels (in ng/ml) in wild type and ICE-deficient (ICE KO) mice treated with vehicle alone or dexamethasone (4 mg/kg) 30 minutes after LPS in the LPS/*P. acnes* septic shock model, demonstrating that the ICE-deficient mice, but not wild type mice, exhibit suppression of TNF α production and hence are steroid responsive.

Figure 2 is a bar graph showing serum TNF α levels (in ng/ml) in wild type (solid bars) and ICE-deficient (hatched bars) mice pretreated with vehicle alone or decreasing amounts of dexamethasone (0.05, 0.005 or 0.0005 mg/kg) in the LPS/*P. acnes* septic shock model, demonstrating that the ICE-deficient mice maintain steroid responsiveness to decreasing steroid dosages in contrast to the wild type mice.

Figure 3 is a bar graph showing LPS-induced serum IL-12 (in pg/ml) in B6 mice pretreated with vehicle alone or with the phosphodiesterase IV inhibitor, rolipram, demonstrating that treatment with the phosphodiesterase IV inhibitor inhibits production of IL-12.

Figure 4 is a bar graph showing serum TNF α levels (in ng/ml) in B6 mice treated with vehicle alone (saline) or an ICE inhibitor (Ac-YVAD-CHO), in combination with dexamethasone treatment, in the LPS/*P. acnes* septic shock model.

Figure 5 is a bar graph showing serum IL-6 levels (in ng/ml) in B6 mice treated with vehicle alone (saline) or an ICE inhibitor (Ac-YVAD-CHO), in combination with dexamethasone treatment, in the LPS/*P. acnes* septic shock model.

Figure 6 is a bar graph showing serum IL-1 β levels (in ng/ml) in B6 mice treated with vehicle alone (saline) or an ICE inhibitor (Ac-YVAD-CHO), in combination with dexamethasone treatment, in the LPS/*P. acnes* septic shock model.

Detailed Description of the Invention

This invention is based, at least in part, upon the discovery that ICE deficient mice, in contrast to wild type control mice, are responsive to corticosteroids after LPS challenge in a septic shock model (see Example 1). Moreover, the ICE deficient mice show increased sensitivity to low doses of corticosteroids compared to wild type control mice, when corticosteroid treatment is given before LPS challenge in the septic shock model (see Example 2). The invention further is based, at least in part, upon the discovery that

depletion of NK/NK-like cells in LPS-challenged wild type mice leads to substantially decreased IFN- γ production (compared to control untreated mice) and to substantially increased survival rates (see Example 10).

5 It has previously been described that administration of interferon- γ (IFN- γ) can overcome corticosteroid suppression of TNF α biosynthesis by murine macrophages (Leudke, C.E. and Cerami, A. (1990) *J. Clin. Invest.* 86:1234-1240). Moreover, ICE and other caspase family proteases can cleave the precursor form of IL-18 to its mature, active form (see Example 4). Although not intending to be limited by mechanism, the ability to confer corticosteroid responsiveness by inhibiting ICE activity in a subject, in accordance
10 with the present invention, is thought to result from inhibition of IL-18 processing by ICE such that production of mature IL-18 is inhibited, thereby leading to decreased production of IFN- γ in the subject. Moreover, IL-18 in conjunction with IL-12 stimulates NK/NK-like cells to make more IFN- γ . Thus, NK/NK-like cells are thought to form a positive feedback loop in the production of IFN- γ , which can be downmodulated by depletion or elimination
15 of the NK/NK-like cells.

In view of the foregoing, the invention broadly provides methods and compositions for modulating responsiveness to corticosteroids in which a target that regulates production of IFN- γ is antagonized in a subject. This target that regulates production of IFN- γ , and which is antagonized, can be IL-18 (which can be antagonized, for example, indirectly by
20 inhibiting ICE activity or directly by use of an anti-IL-18 antibody). Alternatively, another factor that regulates production of IFN- γ , such as IL-12, can be antagonized to thereby modulate corticosteroid responsiveness in the subject. Still further, an agent that depletes or eliminates NK/NK-like cells to thereby inhibit IFN- γ production can be used to modulate corticosteroid responsiveness in the subject.

25

So that the invention may be more readily understood, a number of terms are first defined.

As used herein, the term "corticosteroid" refers to a class of therapeutic agents useful in treatment of inflammatory conditions, including those resulting from infection,
30 transplant rejection and autoimmune disorders. Corticosteroids include those that are naturally occurring, synthetic, or semi-synthetic in origin, and are characterized by the presence of a steroid nucleus of four fused rings, for example, as found in cholesterol, dihydroxycholesterol, stigmasterol, and lanosterol structures. Corticosteroid drugs include cortisone, cortisol, hydrocortisone (11 β 17-dihydroxy-21-(phosphonoxy)-pregn-4-ene3,20-
35 dione disodium), dihydroxycortisone, dexamethasone (21-(acetyloxy)-9-fluoro-11 β ,17-dihydroxy-16 α -methylpregna-1,4-diene-3,20-dione), and highly derivatized steroid drugs such as beconase (beclomethasone dipropionate, which is 9-chloro-11 β ,17,21, trihydroxy-16 β -methylpregna-1, 4 diene-3, 20-dione 17,21-dipropionate). Other examples of

corticosteroids include flunisolide, prednisone, prednisolone, methylprednisolone, triamcinolone, deflazacort and betamethasone.

The term "target that regulates production of IFN- γ " is intended to include chemical factors (e.g., cytokines, enzymes and the like) and cells that directly or indirectly control the synthesis of IFN- γ in a subject. Examples of factors that regulate the production of IFN- γ include IL-18 (see e.g., Okamura, H. *et al.* (1995) *Nature* 378:88-91; Ushio, S. *et al.* (1996) *J. Immunol.* 156:4274-4279) and interleukin-12 (IL-12)(see e.g., Schoenhaut, D. *et al.* (1992) *J. Immunol.* 148:3433; PCT Publication WO 90/05147; European Patent Application EP 433 827 A2). Examples of cells that regulate IFN- γ production include NK and NK-like cells.

As used herein, agents that "antagonize" a factor are intended to include agents that inhibit the activity of the factor and agents that downregulate (*i.e.*, inhibit) the synthesis or production of the factor.

The term "IL-18" refers to a cytokine having an amino acid sequence as disclosed in Okamura, H. *et al.* (1995) *Nature* 378:88-91 (mouse) or Ushio, S. *et al.* (1996) *J. Immunol.* 156:4274-4279 (human), and other mammalian homologues thereof. The cytokine IL-18 has also been referred to in the art as Interferon γ Inducing Factor (IGIF) and IL-1 γ .

The term "IL-18 antagonist" is intended to include agents that inhibit the synthesis or production of IL-18, agents that inhibit the activity of IL-18 once synthesized, agents that inhibit the interaction of IL-18 with an IL-18 receptor and agents that inhibit the activity of an IL-18 receptor. Examples of IL-18 antagonists include inhibitors of caspase family proteases (e.g., ICE inhibitors) and antibodies, antibody fragments and engineered binding proteins that bind to either IL-18 or an IL-18 receptor.

The term "interleukin-12 (IL-12)" refers to a cytokine having an amino acid sequence as disclosed in Schoenhaut, D. *et al.* (1992) *J. Immunol.* 148:3433, PCT Publication WO 90/05147; and European Patent Application EP 433 827 A2, and other mammalian homologues thereof.

The term "IL-12 antagonist" is intended to include agents that inhibit the synthesis or production of IL-12, agents that inhibit the activity of IL-12 once synthesized, agents that inhibit the interaction of IL-12 with an IL-12 receptor and agents that inhibit the activity of an IL-12 receptor. Examples of IL-12 antagonists include antibodies, antibody fragments and engineered binding proteins that bind to either IL-12 or an IL-12 receptor, agents that stimulate intracellular production of cAMP in cells that produce IL-12 (such as phosphodiesterase IV inhibitors or beta-2 agonists) and agents that inhibit STAT4.

The term "caspase family protease" is intended to include members of the caspase proteases as described in Alnemri, E. *et al.* (1996) *Cell* 87:171, including caspase-1 (ICE), caspase-2 (ICH-1), caspase-3 (CPP32, Yama, apopain), caspase-4 (TX, ICH-2, ICE_{rel}-II), caspase-5 (ICE_{rel}-III, TY), caspase-6 (Mch2), caspase-7 (Mch3, ICE-LAP3, CMH-1),

caspase-8 (MACH, FLICE, Mch5), caspase-9 (ICE-LAP6, Mch6) and caspase-10 (Mch4). Furthermore, a "caspase family protease" is intended to include any protein that shares greater than 20% amino acid sequence identity with ICE in the active domains of the protease (*i.e.*, active domains of the p10 and p20 subunits of ICE), contains the peptide
5 sequence glutamine-alanine-cysteine-X-glycine (QACXG), wherein the cysteine (C) is the catalytically active cysteine residue and X denotes any amino acid, and contains the sequence serine-histidine-glycine (SHG), located N-terminal to the QACXG motif, in which the histidine (H) is the catalytically essential histidine residue. Caspase family proteases typically demonstrate a strong preference for hydrolysis of peptide bonds
10 immediately following an acidic amino acid (*i.e.*, aspartic acid or glutamic acid).

Caspase family proteases are known in humans and other organisms including mice and *Caenorhabditis elegans*. Examples of caspase family proteases include, for example, Ich-1 (Wang, L. *et al.* (1994) *Cell* 78:739-750); ICH-2 (Kamens, J. *et al.* (1995) *J. Biol. Chem.* 270:15250-15256); Mch2 (Fernandes-Alnemri, T. *et al.* (1995) *Cancer Res.* 55:2737-2742); CPP32 (Fernandes-Alnemri, T. *et al.* (1994) *J. Biol. Chem.* 269:30761-30764); Yama/CPP32 β (Tewari, M. *et al.* (1995) *Cell* 81:801-809); the product of the mouse gene *Nedd2* (Kumar, S. *et al.* (1992) *Biochem. Biophys. Res. Commun.* 185:1155-1161; Kumar, S. *et al.* (1994) *Genes Dev.* 8:1613-1626); the product of the *C. elegans* gene, *ced-3* (Yuan, J. *et al.* (1993) *Cell* 75:641-652); the human protein TX (Faucheu, C., *et al.*, (1995) *EMBO J.* 14:1914-1922); ICE_{relII} and ICE_{relIII} (Munday, N.A. *et al.* (1995) *J. Biol. Chem.* 270:15870-15876).
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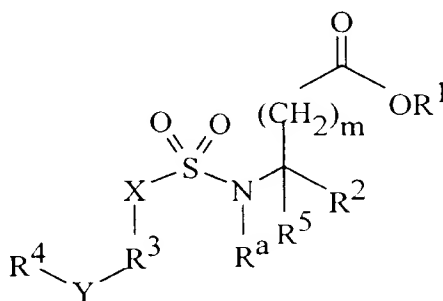
The term "interleukin-1 β converting enzyme (ICE)" is intended to refer to a protease having an amino acid sequence as disclosed in Cerretti, D.P. *et al.* (1992) *Science* 256:97-100 (human) or Nett, M.A. *et al.* (1992) *J. Immunol.* 149:3254-3259 (mouse), and
25 other mammalian homologues thereof.

The term "ICE inhibitor" is intended to include chemical agents that inhibit the proteolytic activity of ICE. Examples of ICE inhibitors are known in the art, including, for example, agents disclosed in U.S. Patent No. 5,585,357 (pyrazolyl derivatives); U.S. Patent No. 5,677,283 (pyrazolyl derivatives); U.S. Patent No. 5,656,627 (inhibitors comprising a
30 hydrogen bonding group, a hydrophobic group and an electronegative group); U.S. Patent No. 5,411,985 (gamma-pyrone-3-acetic acid compounds); U.S. Patent No. 5,430,128 (tripeptidyl derivatives); U.S. Patent No. 5,434,248 (tripeptidyl compounds); U.S. Patent No. 5,565,430 (N,N'-diacylhydrazinoacetic acid compounds); U.S. Patent No. 5,416,013 (peptidyl derivatives); PCT Publication WO 94/21673 (alpha-ketoamide derivatives); PCT
35 Publication WO 97/22619 (N-acylamino compounds); PCT Publication WO 97/22618 (amino acid or di- or tripeptide amide derivatives); PCT Publication WO 95/35308 (inhibitors comprising a hydrogen bonding group, a hydrophobic group and an electronegative group); PCT Publication WO 93/14777 (peptidyl derivatives); PCT

Publication WO 93/16710 (peptidyl derivatives); PCT Publication WO 95/05152 (substituted ketone derivatives); PCT Publication WO 94/03480 (peptidyl 4-amino-2, 2-difluoro-3-oxo-1, 6-hexanedioic acid derivatives); PCT Publication WO 94/00154 (peptidyl derivatives); PCT Publication WO 93/05071 (peptidyl derivatives); European Application
 5 EP 519 748 (peptidyl derivatives); European Application EP 590 650 (cyclopropene derivatives); European Application EP 628 550 (pyridazines); European Application EP 644 198 (alpha-heteroaryloxymethyl ketones); European Application EP 644 197 (peptidic phosphinyloxymethyl ketones); European Patent Application EP 547 699 (peptidyl derivatives); Great Britain Application GB 2,278,276 (gamma-pyrone-3-acetic
 10 compounds); and Canadian Application 2,109,646 (para-nitroanilide peptides). The present invention encompasses use of the ICE inhibitors disclosed in any of the foregoing publications in the methods described herein.

Additional preferred ICE inhibitors for use in the methods of the invention include sulfonamide substituted aspartic acid ICE inhibitors having the formula I:

15



I

wherein

R^1 is hydrogen, C_1 - C_6 alkyl, or benzyl;

R^2 is -CHO, -COR^a, or -CN;

20 each R^a is independently hydrogen or C_1 - C_6 alkyl;

X is a bond, CH_2 , $CH(R^5)$, NH, NR^5 , or O;

R^3 is aryl, substituted-aryl, heteroaryl, substituted-heteroaryl, cycloalkyl, substituted-cycloalkyl, heterocycle, or substituted-heterocycle;

Y is absent, NR^5 , CO, S, O, SO_2 , $-O(CH(R^5))_n$, CHR^5 , NR^5CO , NCR^5 , $CONR^5$, $OCHR^5$,
 25 CHR^5O , $SCHR^5$, CHR^5S , SO_2NR^5 , C_1 - C_6 alkyl, NR^5SO_2 , CH_2CHR^5 ,
 CHR^5CH_2 , $COCH_2$, or CH_2CO ;

R^4 is absent, aryl, substituted-aryl, C_1 - C_6 alkyl, heteroaryl, substituted-heteroaryl, cycloalkyl, C_1 - C_6 alkyl, substituted-cycloalkyl, heterocycloalkyl, or substituted-heterocycloalkyl;

30 each R^5 is independently hydrogen, C_1 - C_6 alkyl, aryl, $-(CH_2)_n$ aryl, or $-(CH_2)_n$ cycloalkyl;

each n is independently 0 to 5, m is 1 or 2, and the pharmaceutically acceptable salts, esters, amides, and prodrugs thereof.

In one embodiment of the invention, R² is CHO.

In another embodiment of the invention, R¹ is hydrogen.

5 In another embodiment of the invention, R^a is hydrogen.

In another embodiment of the invention, X is a bond.

In another embodiment of the invention, R³ is phenyl or substituted phenyl.

In another embodiment of the invention, Y is a bond.

In another embodiment of the invention, Y is O.

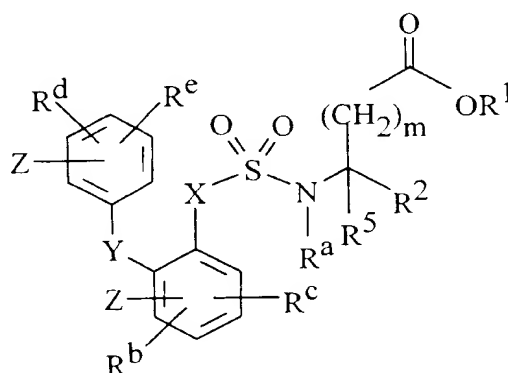
10 In another embodiment of the invention, Y is CH₂.

In another embodiment of the invention, R⁴ is phenyl or substituted phenyl.

In another embodiment of the invention, R² is CHO, R^a is H, R¹ is hydrogen, X is a bond, R³ and R⁴ are phenyl or substituted phenyl, and Y is a bond, CH₂, or O.

In another embodiment of the invention, m is 1 and R⁵ is hydrogen.

15 Other preferred sulfonamide substituted ICE inhibitors have the Formula II



II

wherein

R¹ is hydrogen, C₁-C₆alkyl, or benzyl;

R² is -CHO, -COR^a, or -CN;

20 each R^a is independently hydrogen or C₁-C₆alkyl;

X is a bond, CH₂, CHR⁵, NH, NR⁵, or O;

Y is a bond, NR⁵, CO, S, O, SO₂, CHR⁵, NR⁵CO, CONR⁵, OCHR⁵, CHR⁵O, -O(CHR⁵)_n, SCHR⁵, CHR⁵S, SO₂NR⁵, NR⁵SO₂, CH₂CHR⁵, CHR⁵CH₂, COCH₂, or CH₂CO;

25 each R⁵ is independently hydrogen, C₁-C₆alkyl, aryl, or -(CH₂)_naryl;

each n is independently 0 to 5;

m is 1 or 2;

Each Z is independently hydrogen, or an aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocycle, or substituted heterocycle group that is fused to the phenyl group that contains Z as a substituent;

R^b, R^c, R^d, and R^e are each independently hydrogen, C₁-C₆alkyl, C₁-C₆alkoxy, -OH,

- 5 C₁-C₆ thioalkoxy, halogen, trifluoromethyl, dialkylamino, -NO₂, -CN, -CF₃, -CO₂alkyl, -SO₃H, -CHO, -COalkyl, -CONH-alkyl, -CONHR^q, -CON(alkyl)₂, -(CH₂)_n-NH₂, -(CH₂)_n-NH-alkyl, -NHR^q, -NHCOR^q, -(CH₂)_nOH, -(CH₂)_nCONH₂, or -(CH₂)_nCO₂H; and

- 10 R^q is hydrogen or C₁-C₆alkyl, and the pharmaceutically acceptable salts, esters, amides, and prodrugs thereof.

In another embodiment with respect to the compounds of Formula II, R¹ is hydrogen.

In another embodiment with respect to the compounds of Formula II, R² is CHO.

- 15 In another embodiment with respect to the compounds of Formula II, R^a is hydrogen.

In another embodiment with respect to the compounds of Formula II, X is a bond.

In another embodiment with respect to the compounds of Formula II, Y is a bond, O, or CH₂.

- 20 In another embodiment with respect to the compounds of Formula II, R^b and R^c are hydrogen.

In another embodiment with respect to the compounds of Formula II, wherein R^b, R^c, and R^d are hydrogen and R^e is C₁-C₆ alkyl.

- In another preferred embodiment with respect to the compounds of Formula II, R^b or R^c is located at the para position of the phenyl ring with respect to X and R^b or R^c is -OCH₃.

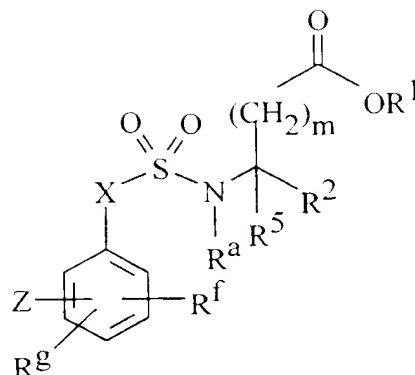
25 In another embodiment with respect to the compounds of Formula II, m is 1 and R⁵ is hydrogen.

Preferred compounds include:

- 30 3-(Biphenyl-2-sulfoamino)-4-oxo-butyric acid;
3-(2-Benzyl-benzenesulfonylamino)-4-oxo-butyric acid;
4-Oxo-3-(2-phenoxy-benzenesulfonylamino)-butyric acid;
4-Oxo-3-(2-p-tolyloxy-benzenesulfonylamino)-butyric acid;
3-[2-(4-Isopropyl-phenoxy)-benzenesulfonylamino]-4-oxo-butyric acid;
4-Oxo-3-(2-m-tolyloxy-benzenesulfonylamino)-butyric acid;
35 3-[2-(3-Isopropyl-phenoxy)-benzenesulfonylamino]-4-oxo-butyric acid; and
3-(4'-Methyl-biphenyl-2-sulfonylamino)-4-oxo-butyric acid.

Other ICE inhibitors include compounds of the Formula III

-12-



III

wherein

R^1 is hydrogen, C_1 - C_6 alkyl, or benzyl;

R^2 is -CHO, -COR^a, or -CN;

5 each R^a is independently hydrogen or C_1 - C_6 alkyl;

X is a bond, CH_2 , CHR^5 , NH, NR^5 , or O;

R^5 is hydrogen, C_1 - C_6 alkyl, aryl, or $-(CH_2)_n$ aryl;

each n is independently 0 to 5;

m is 1 or 2;

10 Z is absent, or an aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocycle, or substituted heterocycle group that is fused to the phenyl group that contains Z as a substituent;

R^f , R^g , are each independently hydrogen, C_1 - C_6 alkyl, hydroxy, halogen, trifluoromethyl, dialkylamino, -NO₂, -CN, -CO₂H, -CO₂alkyl, -SO₃H, -CHO, -COalkyl, -CONH₂,
 15 -CONH(CH₂)_naryl, -CONH(CH₂)_n-substituted-aryl, -CONH-alkyl, -CONHR^q,
 -CON(alkyl)₂, $-(CH_2)_n$ -NH₂, $-(CH_2)_n$ -NH-alkyl, -NHR^q, -NHCOR^q, -OR^q, -SR^q,
 or $-(CH_2)_n$ aryl; and

R^q is hydrogen or C_1 - C_8 alkyl, and the pharmaceutically acceptable salts, esters, amides, and prodrugs thereof.

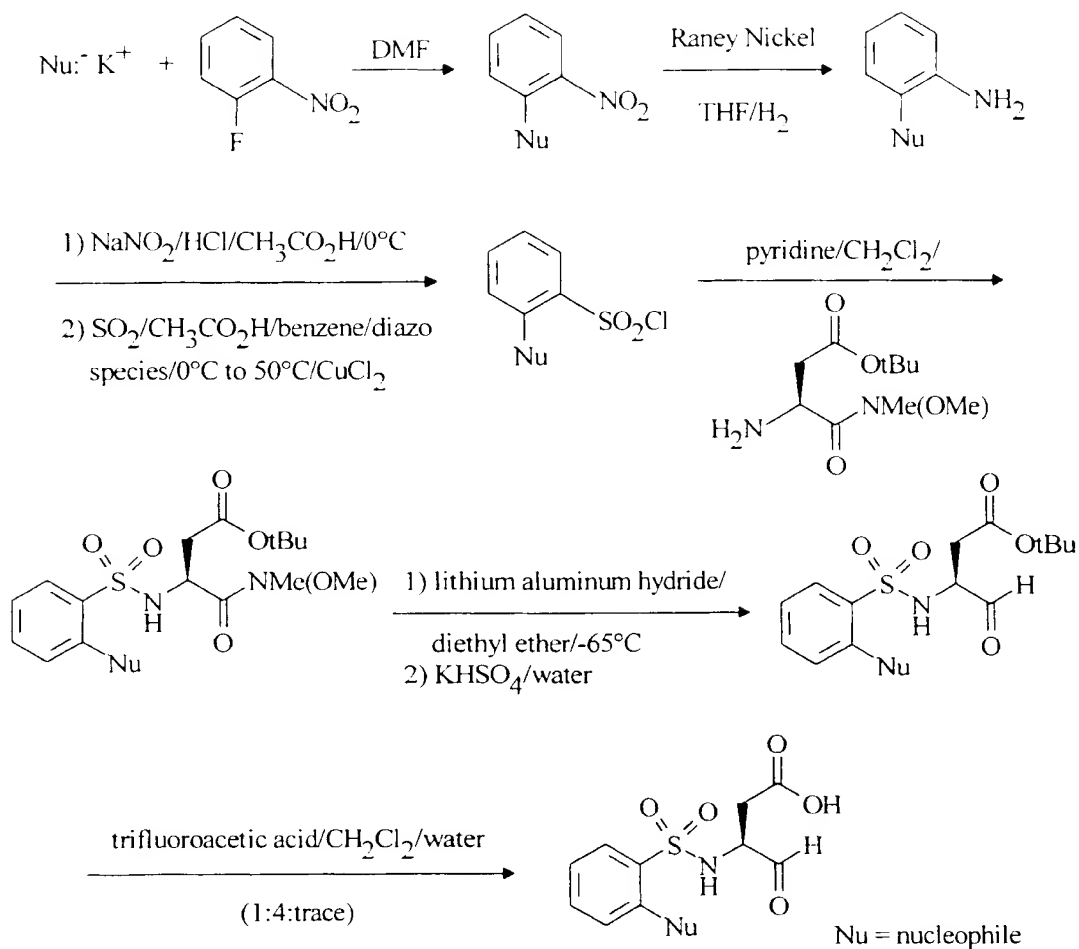
20 In a preferred embodiment of the compounds of Formula III, R^f is ortho to X on the phenyl ring, and R^g is hydrogen.

In a preferred embodiment of the compounds of Formula III, Z is hydrogen, m is 1, R^5 is hydrogen, and R^a is hydrogen.

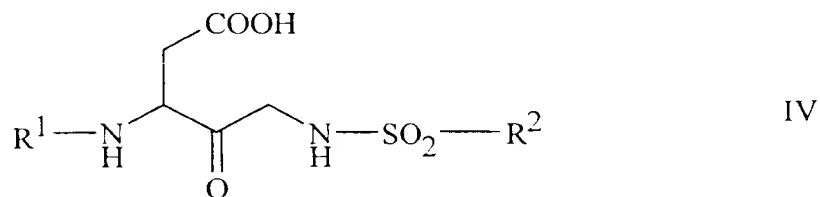
In a preferred embodiment of the compounds of Formula III, the compound is
 25 3-benzenesulfonylamino-4-oxo-butyric acid.

The above-described sulfonamide substituted aspartic acid ICE inhibitors can be made generally as follows:

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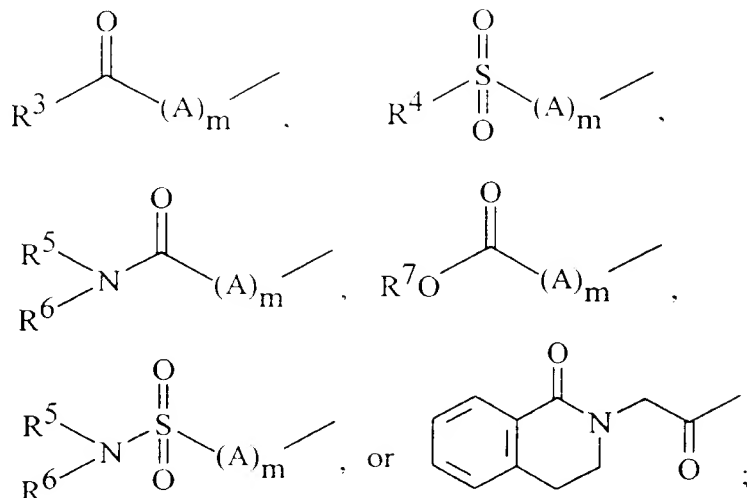


Other sulfonamide ICE inhibitors that can be used in the invention are compounds
 5 of Formula IV:



wherein R^1 is

-14-



R^3 is hydrogen,

C_1 - C_6 alkyl,

$-(CH_2)_n$ aryl, or

5 $-(CH_2)_n$ heteroaryl;

R^4 is C_1 - C_6 alkyl,

$-(CH_2)_n$ aryl, or

$-(CH_2)_n$ heteroaryl;

R^5 and R^6 are each independently hydrogen,

10 C_1 - C_6 alkyl,

$-(CH_2)_n$ aryl, or

$-(CH_2)_n$ heteroaryl;

R^7 is C_1 - C_6 alkyl,

$-(CH_2)_n$ aryl, or

15 $-(CH_2)_n$ heteroaryl;

each n is 0 to 6;

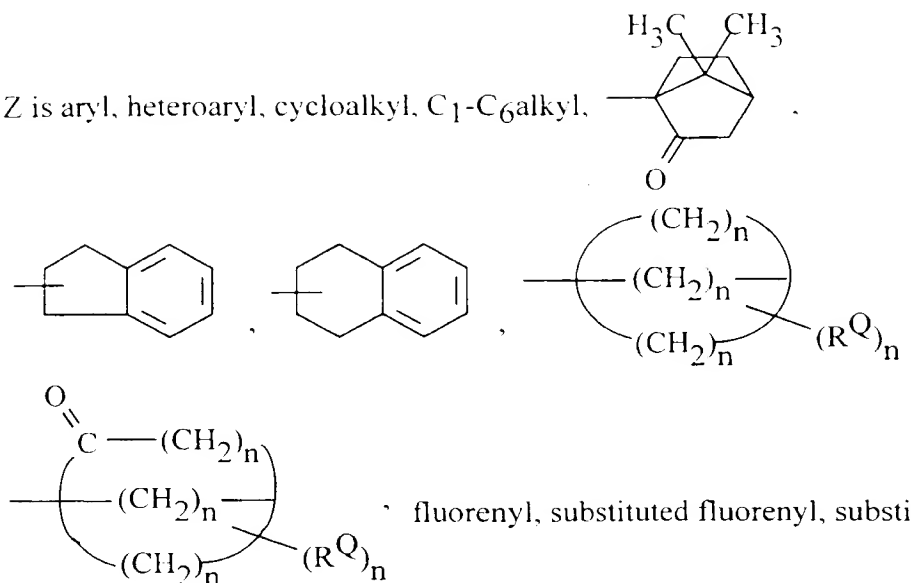
each m is 0, 1, 2, or 3;

A is alanine, leucine, isoleucine, proline, phenylalanine, glycine, tyrosine, serine, threonine, tryptophan, cysteine, methionine, valine, asparagine, glutamine, aspartic acid, lysine,

20 glutamic acid, arginine, or histidine;

R^2 is $-(CH_2)_n-Z$; and

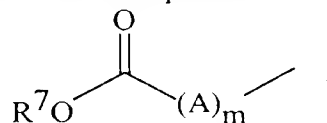
Z is aryl, heteroaryl, cycloalkyl, C₁-C₆alkyl,



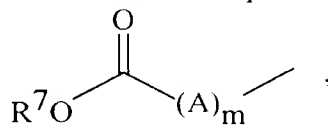
heteroaryl, or substituted cycloalkyl, and the pharmaceutically acceptable salts, esters,

5 amides, and prodrugs thereof.

In a preferred embodiment of the compounds of Formula IV, R¹ is

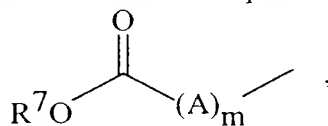


In another preferred embodiment of the compounds of Formula IV, R¹ is



10 m is 0, and R⁷ is -(CH₂)_n aryl.

In another preferred embodiment of the compounds of Formula IV, R¹ is



m is 0, and R⁷ is -CH₂ aryl.

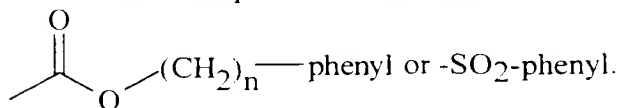
In another preferred embodiment of the compounds of Formula IV, R² is -(CH₂)_n

15 aryl.

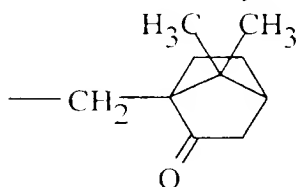
In another preferred embodiment of the compounds of Formula IV, aryl is phenyl or naphthyl.

In another preferred embodiment of the compounds of Formula IV, R² is -(CH₂)_n-cycloalkyl.

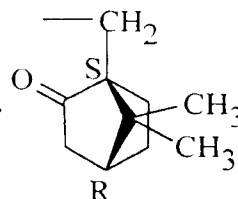
20 In another preferred embodiment of the compounds of Formula IV, R¹ is



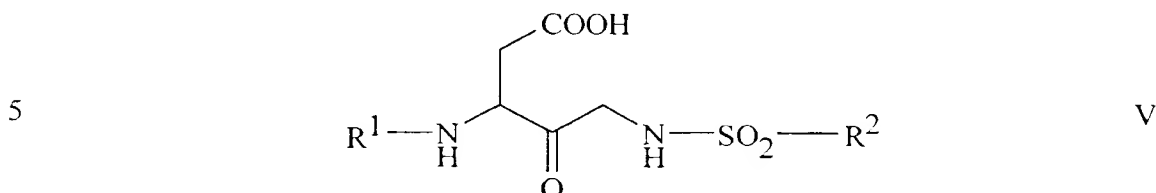
In another preferred embodiment of the compounds of Formula IV, R^2 is



In another preferred embodiment of Formula IV, R^2 is

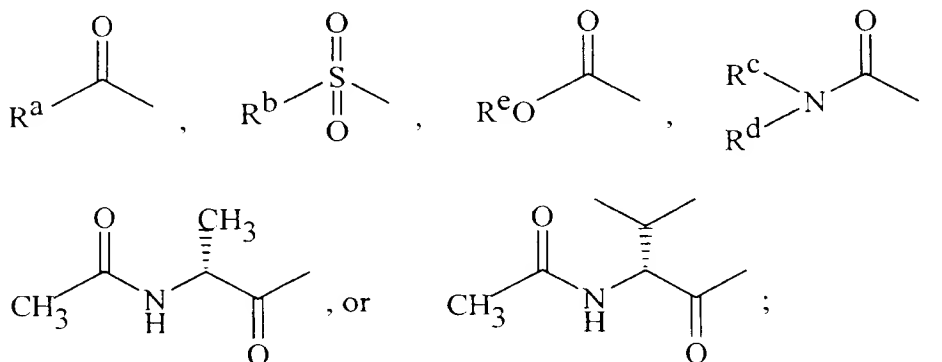


Other sulfonamide ICE inhibitors include compounds of the Formula V



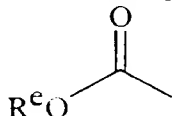
wherein R^2 is $-\text{CH}_2\text{CH}_2-$ aryl, $-\text{CH}_2-$ cycloalkyl, $-\text{CH}_2\text{CH}_2-$ cycloalkyl, or $-\text{CH}_2\text{CH}_2-$ heteroaryl;

R^1 is

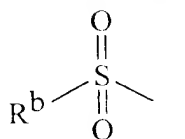


- 10 R^a is $-(\text{CH}_2)_n-$ aryl or $-(\text{CH}_2)_n$ heteroaryl;
 R^b is aryl or heteroaryl;
 R^c is $-\text{CH}_2$ aryl or aryl;
 R^d is hydrogen or C_1 - C_6 alkyl;
 R^e is $-\text{CH}_2$ aryl or $-\text{CH}_2$ heteroaryl; and the pharmaceutically acceptable salts, esters,
 15 amides, and prodrugs thereof.

In a preferred embodiment of the compounds of Formula V R^1 is



In another preferred embodiment of the compounds of Formula V, R¹ is



In another preferred embodiment of the compounds of Formula V, R^e is -(CH₂)_n aryl.

5 In another preferred embodiment of the compounds of Formula V, aryl is phenyl or naphthyl.

In another preferred embodiment of the compounds of Formula V, R^b is aryl.

Preferred compounds include:

- 10 3-Benzyloxycarbonylamino-4-oxo-5-(2-phenoxy-ethanesulfonylamino)-pentanoic acid;
- 3-Benzyloxycarbonylamino-4-oxo-5-(3-phenyl-propane-1-sulfonylamino)-pentanoic acid;
- 3-Benzenesulfonylamino-4-oxo-5-(2-phenylethane-1-sulfonylamino)-pentanoic acid;
- 15 5-Benzenesulfonylamino-3-benzyloxycarbonylamino-4-oxo-pentanoic acid;
- 3-Benzyloxycarbonylamino-5-methanesulfonylamino-4-oxo-pentanoic acid;
- 3-Benzyloxycarbonylamino-5-(naphthalene-1-sulfonylamino)-4-oxo-pentanoic acid;
- 3-Benzyloxycarbonylamino-5-(2-cyclohexyl-ethanesulfonylamino)-4-oxo-pentanoic acid;
- 20 3-Benzyloxycarbonylamino-5-(2-naphthalen-1-yl-ethanesulfonylamino)-4-oxo-pentanoic acid;
- 3-Benzyloxycarbonylamino-5-(7,7-dimethyl-2-oxo-bicyclo[2.2.1]hept-1-(R)-ylmethanesulfonylamino)-4-oxo-pentanoic acid;
- 25 3-Benzyloxycarbonylamino-5-(indan-1-ylmethanesulfonylamino)-4-oxo-pentanoic acid;
- 3-Benzyloxycarbonylamino-5-(9-fluoro-9H-fluoren-9-ylmethanesulfonylamino)-4-oxo-pentanoic acid;
- 3-Benzyloxycarbonylamino-5-(7,7-dimethyl-2-oxo-bicyclo[2.2.1]hept-1-(S)-ylmethanesulfonylamino)-4-oxo-pentanoic acid;
- 30 3-[2-(2-Benzyloxycarbonylamino-3-methyl-butyrylamino)-propionylamino]-4-oxo-5-(2-phenyl-ethanesulfonylamino)-pentanoic acid;
- 3-[2-(2-Benzyloxycarbonylamino-4-carboxy-butyrylamino)-3-methyl-butyrylamino]-4-oxo-5-(2-phenyl-ethanesulfonylamino)-pentanoic acid;
- 35 3-{2-[4-Carboxy-2-(3-phenyl-propionylamino)-butyrylamino]-3-methyl-butyrylamino}-4-oxo-5-(2-phenyl-ethanesulfonylamino)-pentanoic acid;

3-(2-{2-[2-Acetylamino-3-(4-hydroxy-phenyl)-propionylamino]-4-carboxy-butylamino}-3-methyl-butylamino)-4-oxo-5-(2-phenyl-ethanesulfonylamino)-pentanoic acid;

5 3-(2-Acetylamino-3-methyl-butylamino)-5-(7,7-dimethyl-2-oxo-bicyclo[2.2.1]hept-1-(S)-ylmethanesulfonylamino)-4-oxo-pentanoic acid;

3-(2-Acetylamino-propylamino)-5-(7,7-dimethyl-2-oxo-bicyclo[2.2.1]hept-1-(S)-ylmethanesulfonylamino)-4-oxo-pentanoic acid;

10 3-[2-(2-Benzoyloxycarbonylamino-3-methyl-butylamino)-propionylamino]-5-(7,7-dimethyl-2-oxo-bicyclo[2.2.1]hept-1-ylmethanesulfonylamino)-4-oxo-pentanoic acid;

3-{2-[4-Carboxy-2-(3-phenyl-propionylamino)-butylamino]-3-methyl-butylamino}-5-(7,7-dimethyl-2-oxo-bicyclo[2.2.1]hept-1-ylmethanesulfonylamino)-4-oxo-pentanoic acid;

15 3-(2-{2-[2-Acetylamino-3-(4-hydroxy-phenyl)-propionylamino]-4-carboxy-butylamino}-3-methyl-butylamino)-5-(7,7-dimethyl-2-oxo-bicyclo[2.2.1]hept-1-ylmethanesulfonylamino)-4-oxo-pentanoic acid;

3-[2-(2-Benzoyloxycarbonylamino-4-carboxy-butylamino)-3-methyl-butylamino]-5-(7,7-dimethyl-2-oxo-bicyclo[2.2.1]hept-1-ylmethanesulfonylamino)-4-oxo-pentanoic acid;

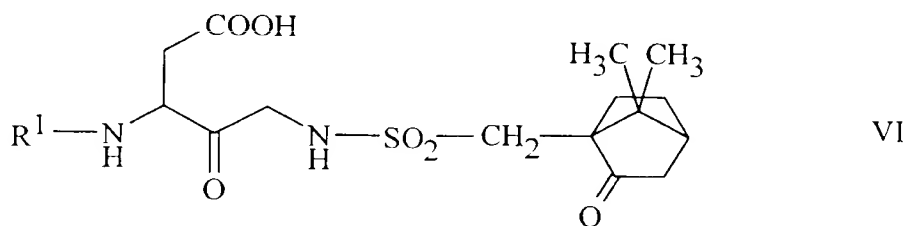
20 3-(1,2,3,4-tetrahydro-1-oxo-isoquinoline-2-yl)-acetanino-5-benzenesulfonylamino-4-hydroxy-pentanoic acid;

(S)-5-(Bicyclo[2.2.1]hept-1-ylmethanesulfonylamino)-4-oxo-3-[2-(1-oxo-3,4-dihydro-1H-isoquinolin-2-yl)-acetylamino]-pentanoic acid;

(S)- 4-Oxo-3-[2-(1-oxo-3,4-dihydro-1H-isoquinolin-2-yl)-acetylamino]-5-(2-phenyl-ethanesulfonylamino)-pentanoic acid; and

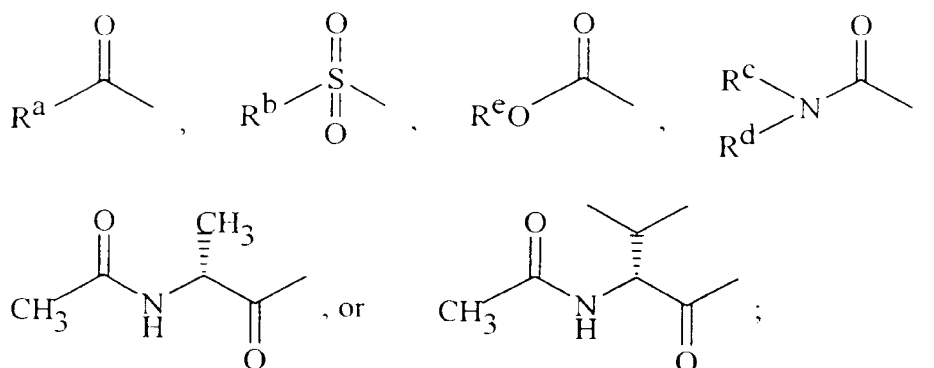
25 4-Oxo-3-[2-(1-oxo-3,4-dihydro-1H-isoquinolin-2-yl)-acetylamino]-5-phenylmethanesulfonylamino-pentanoic acid.

Other sulfonamide ICE inhibitors include compounds of the Formula VI:



wherein

R¹ is



R^a is $-(CH_2)_n$ - aryl or $-(CH_2)_n$ heteroaryl;

R^b is aryl or heteroaryl;

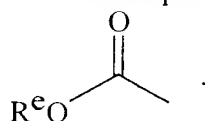
5 R^c is $-CH_2$ aryl or aryl;

R^d is hydrogen or C₁-C₆ alkyl;

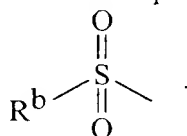
R^e is $-CH_2$ aryl or $-CH_2$ heteroaryl; and the pharmaceutically acceptable salts, esters, amides, and prodrugs thereof.

In a preferred embodiment of the compounds of Formula VI, R¹ is

10



In a preferred embodiment of the compounds of Formula VI, R¹ is



In another preferred embodiment of the compounds of Formula VI, R^e is $-(CH_2)_n$ aryl.

15

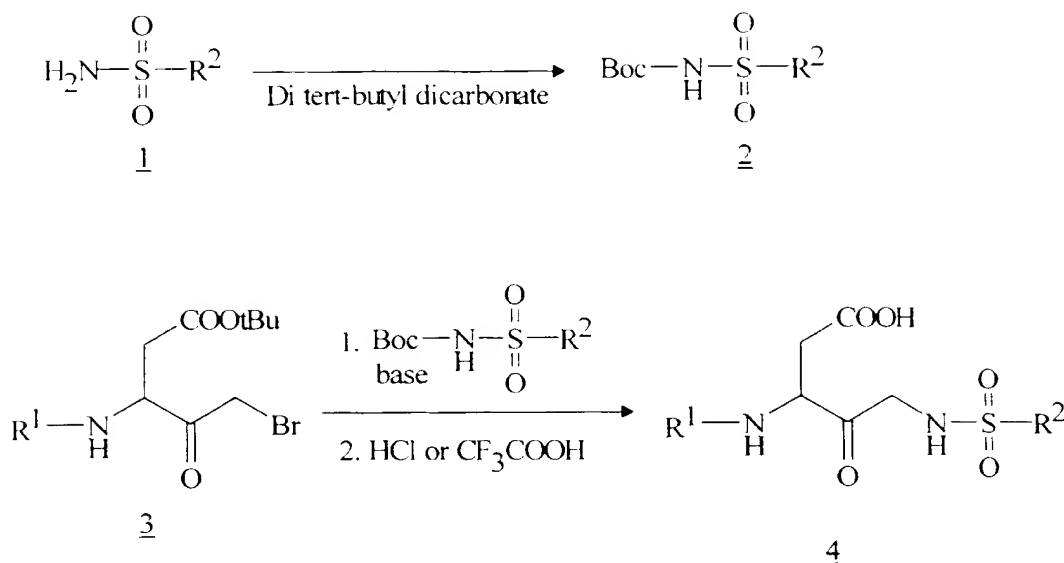
In another preferred embodiment of the compounds of Formula VI, aryl is phenyl or naphthyl.

In another preferred embodiment of the compounds of Formula VI, R^b is aryl.

20

The above-described compounds of Formulas IV, V or VI can be prepared generally by converting the appropriate starting sulfonamide 1 to Boc sulfonamide 2 using a reagent such as di-tert-butyl dicarbonate. Boc sulfonamide 2 may then be reacted with the appropriately substituted aspartic acid bromomethylketone β tert-butyl ester 3 in the presence of a base, followed by treatment with acid to give the desired product 4.

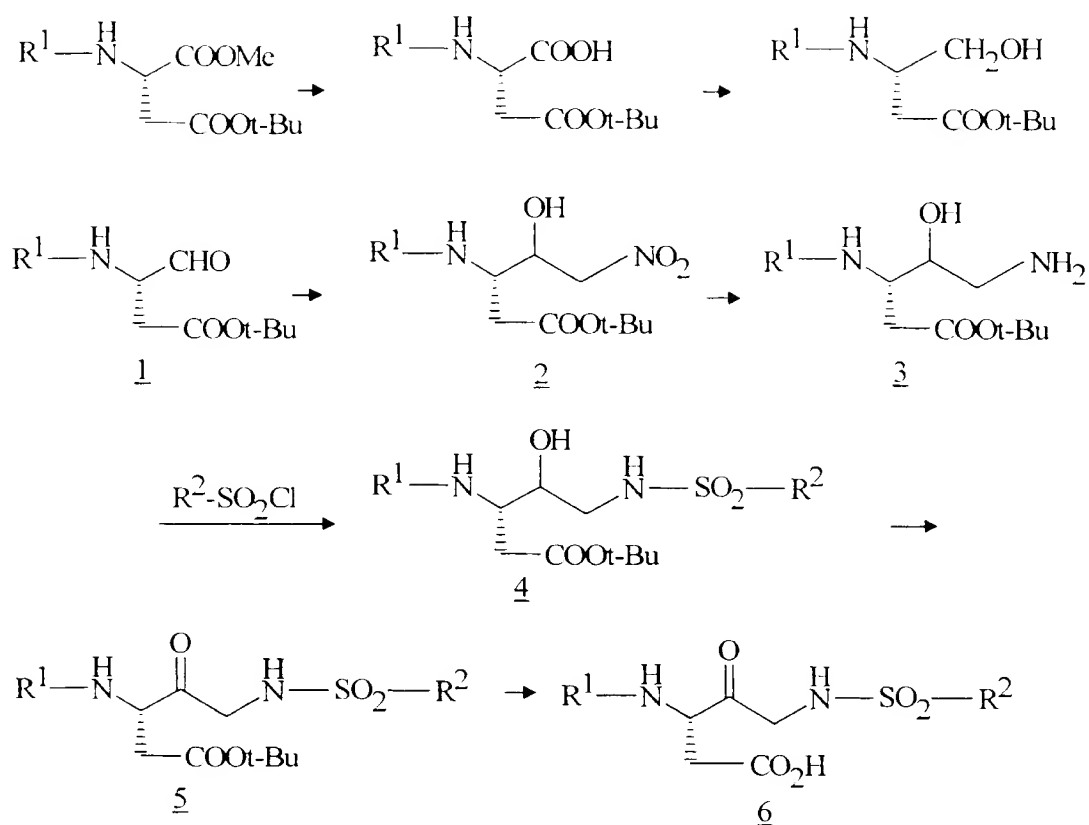
Scheme 1



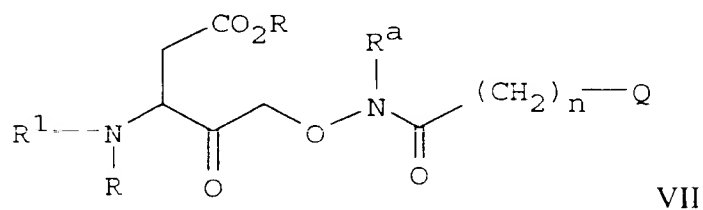
- Alternatively, compounds of Formulas IV, V or VI can be prepared generally by reaction of the appropriately substituted aspartic acid aldehyde 1 with nitromethane in the presence of a base such as potassium tert-butoxide to give nitro alcohol 2. Reduction of 2 to the amine 3, followed by reaction with the appropriate sulfonyl chloride gives 4 which may be oxidized to the ketone 5 with a reagent such as Dess Martin periodinane or by a Swern oxidation. Acidic deprotection of the t-butyl ester with HCl or trifluoroacetic acid gives the desired product 6.

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Scheme 2



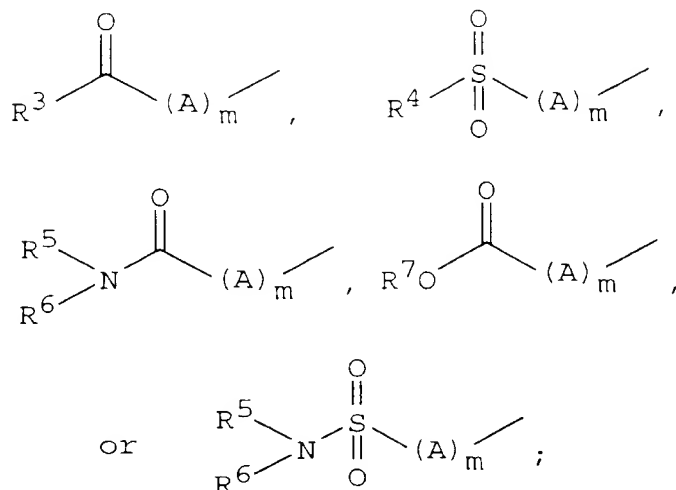
5 Still other ICE inhibitor compounds that can be used in the invention include hydroxamate compounds, including compounds of the formula VII:



10

wherein R^1 is

-22-



each R is independently hydrogen or C₁-C₆alkyl;

- 5 R³ is hydrogen, C₁-C₆alkyl, -(CH₂)_naryl, -(CH₂)_nheteroaryl, -(CH₂)_p-X-aryl, or -(CH₂)_p-X-heteroaryl;

R⁴ is C₁-C₆alkyl, -(CH₂)_naryl, -(CH₂)_nheteroaryl, -(CH₂)_j-X-aryl, or -(CH₂)_j-X-heteroaryl;

R⁵ and R⁶ are each independently hydrogen, C₁-C₆alkyl, -(CH₂)_naryl, -(CH₂)_nheteroaryl, -(CH₂)_j-X-aryl, or -(CH₂)_j-X-heteroaryl;

- 10 R⁷ is C₁-C₆alkyl, -(CH₂)_paryl, -(CH₂)_pheteroaryl, -(CH₂)_j-X-aryl, or -(CH₂)_j-X-heteroaryl;

each n is independently 0 to 6;

each p is independently 1 to 6;

each j is independently 2 to 6;

each m is 0 to 2;

- 15 A is alanine, valine, serine, threonine, glutamic acid, lysine, arginine, histidine, glutamine, or alpha amino butyric acid;

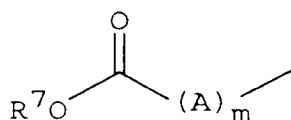
R^a is hydrogen, C₁-C₆alkyl, or -(CH₂)_nphenyl;

X is O or S; and

- 20 Q is C₁-C₆alkyl, -(CH₂)_naryl, -(CH₂)_nheteroaryl, and the pharmaceutically acceptable salts, esters, amides, and prodrugs thereof.

In one embodiment of the compounds of Formula VII, each R is hydrogen.

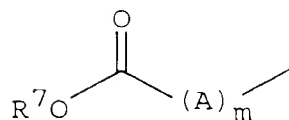
In another embodiment of the compounds of Formula VII, R¹ is



25

and m is 0.

In another embodiment of the compounds of Formula VII, R^1 is



5

m is 0, and R^7 is $-(CH_2)_n$ aryl.

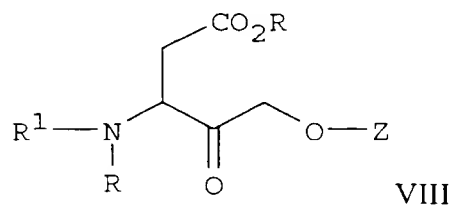
In another embodiment of the compounds of Formula VII, Q is $-(CH_2)_n$ phenyl or $-(CH_2)_n$ naphthyl.

10 In another embodiment of the compounds of Formula VII, R^a is hydrogen or methyl.

In another embodiment of the compounds of Formula VII, Q is $-CH_2$ -phenyl, $-CH_2$ -naphthyl, $-CH_2CH_2$ -phenyl, or $-CH_2CH_2$ -naphthyl.

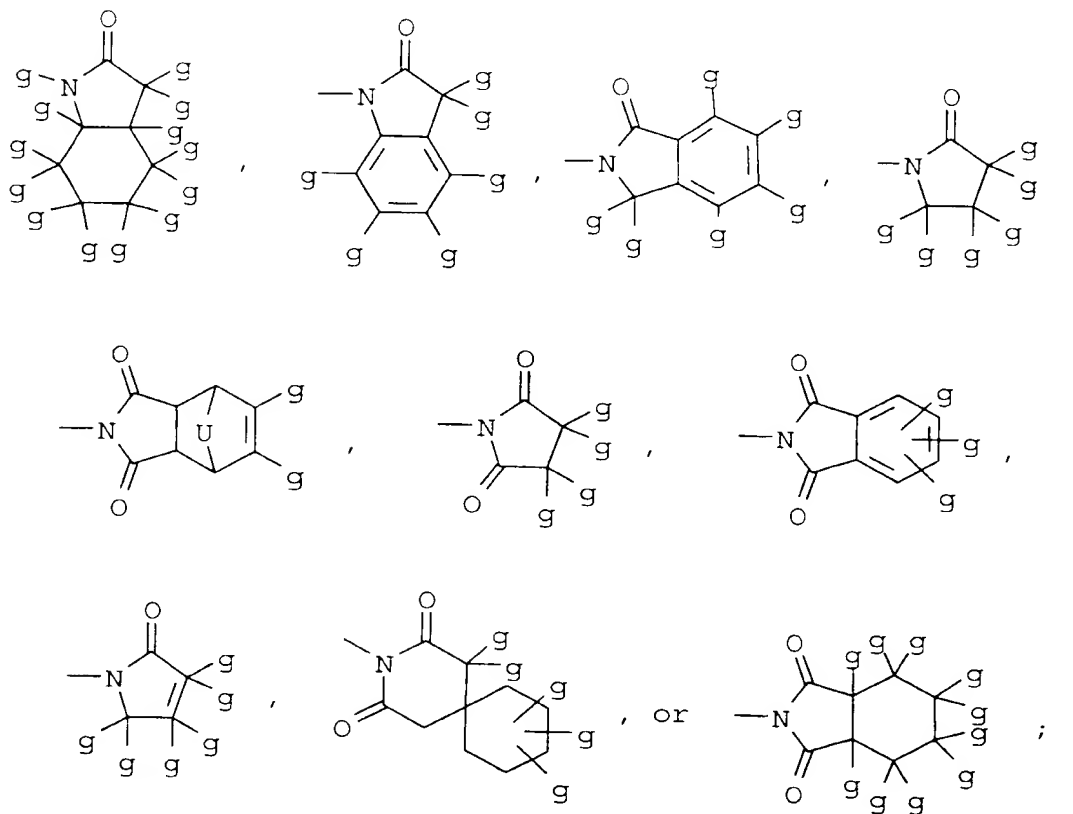
Other hydroxamate ICE inhibitor compounds include compounds having the Formula VIII

15



wherein Z is

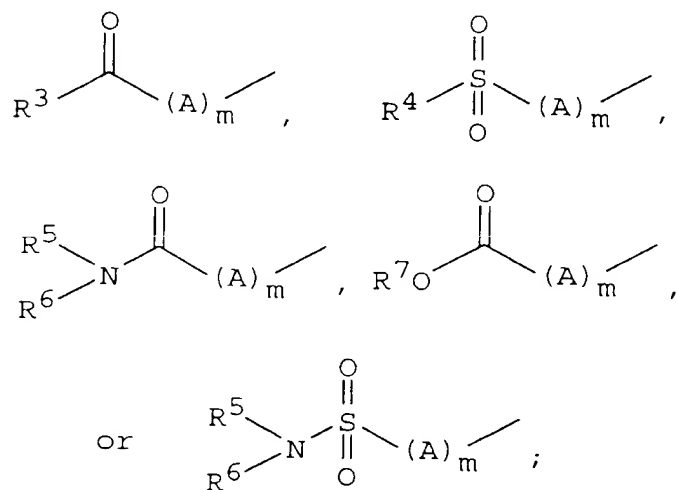
20



each g is independently hydrogen, C₁-C₆alkyl, C₁-C₆alkoxy, -(CH₂)_nCO₂R, -(CH₂)_naryl, -aryl, -(CH₂)_nheteroaryl, or -heteroaryl;

5 U is O or CH₂;

R¹ is



10 each R is independently hydrogen or C₁-C₆alkyl;

R³ is hydrogen, C₁-C₆alkyl, -(CH₂)_naryl, -(CH₂)_nheteroaryl, -(CH₂)_p-X-aryl, or -(CH₂)_p-X-heteroaryl;

R^4 is C_1 - C_6 alkyl, $-(CH_2)_n$ aryl, $-(CH_2)_n$ heteroaryl, $-(CH_2)_j$ -X-aryl, or $-(CH_2)_j$ -X-heteroaryl;
 R^5 and R^6 are each independently hydrogen, C_1 - C_6 alkyl, $-(CH_2)_n$ aryl, $-(CH_2)_n$ heteroaryl, $-(CH_2)_j$ -X-aryl, or $-(CH_2)_j$ -X-heteroaryl;

R^7 is C_1 - C_6 alkyl, $-(CH_2)_p$ aryl, $-(CH_2)_p$ heteroaryl, $-(CH_2)_j$ -X-aryl, or $-(CH_2)_j$ -X-heteroaryl;

5 each n is independently 0 to 6;

each p is independently 1 to 6;

each j is independently 2 to 6;

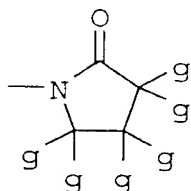
each m is 0 to 2;

A is alanine, valine, serine, threonine, glutamic acid, lysine, arginine, histidine, glutamine,

10 or alpha amino butyric acid; and

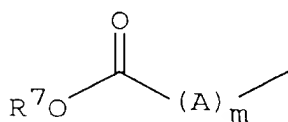
X is O or S, and the pharmaceutically acceptable salts, esters, amides, and prodrugs thereof.

In one embodiment of the compounds of Formula VIII, Z is



15 and each g is hydrogen.

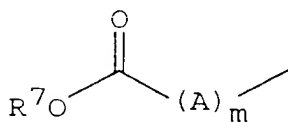
In another embodiment of the compounds of Formula VIII, R^1 is



20

and m is 0.

In one embodiment of the compounds of Formula VIII, R^1 is



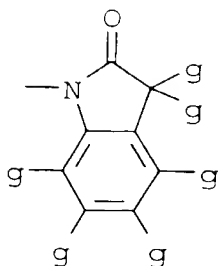
25

m is 0, and R^7 is $-(CH_2)_n$ -aryl.

In one embodiment of the compounds of Formula VIII, R^7 is $-(CH_2)_n$ -phenyl.

In a preferred embodiment of the compounds of Formula VIII, Z is

30



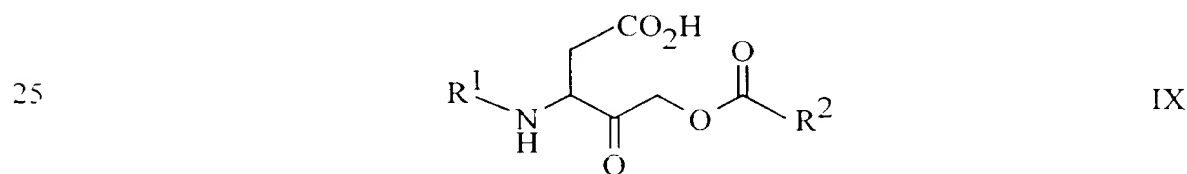
and each g is hydrogen.

Preferred hydroxamate ICE inhibitor compounds include:

- 5 3-Benzyloxycarbonyl-amino-4-oxo-5-phenylacetylaminooxy-pentanoic acid;
- 3-Benzyloxycarbonylamino-4-oxo-5-(2-oxo-pyrrolidin-1-yloxy)-pentanoic acid;
- 3-Benzyloxycarbonylamino-5-(3,5-dioxo-10-oxa-4-aza-tricyclo[5.2.1.0^{2,6}]dec-8-en-4-yloxy)-4-oxo-pentanoic acid;
- 3-Benzyloxycarbonylamino-5-(2-oxo-2,3-dihydro-indol-1-yloxy)-4-oxo-pentanoic acid;
- 10 3-Benzyloxycarbonylamino-5-(7-methoxycarbonylmethyl-2-oxo-octahydro-indol-1-yloxy)-4-oxo-pentanoic acid;
- 3-Benzyloxycarbonylamino-4-oxo-5-(2-oxo-octahydro-indol-1-yloxy)-pentanoic acid;
- 3-[2-(2-Benzyloxycarbonylamino-3-methyl-butyrylamino)-propionylamino]-5-(7-methoxycarbonylmethyl-2-oxo-octahydro-indol-1-yloxy)-4-oxo-pentanoic acid;
- 15 3-[2-(2-benzyloxycarbonylamino-3-methyl-butyrylamino)-propionylamino]-4-oxo-5-(2-oxo-2,3-dihydro-indol-1-yloxy)-pentanoic acid;
- 3-Benzyloxycarbonylamino-5-(2,5-dioxo-pyrrolidin-1-yloxy)-4-oxo-pentanoic acid;
- 3-Benzyloxycarbonylamino-4-oxo-5-(2,2,3-trimethyl-5-oxo-pyrrolidin-1-yloxy)-pentanoic acid;
- 20 3-Benzyloxycarbonylamino-5-(1,3-dioxo-octahydro-isoindol-2-yloxy)-4-oxo-pentanoic acid;
- 3-Benzyloxycarbonylamino-5-(1,3-dioxo-1,3-dihydro-isoindol-2-yloxy)-4-oxo-pentanoic acid;
- 3-Benzyloxycarbonylamino-5-[3-(4-bromo-phenyl)-2,5-dioxo-2,5-dihydro-pyrrol-1-yloxy]-4-oxo-pentanoic acid;
- 25 3-Benzyloxycarbonylamino-5-(3,5-dioxo-4-aza-tricyclo[5.2.1.0^{2,6}]dec-8-en-4-yloxy)-4-oxo-pentanoic acid;
- 3-Benzyloxycarbonylamino-5-(2,4-dioxo-3-aza-spiro[5.5]undec-3-yloxy)-4-oxo-pentanoic acid;
- 30 5-(2-Biphenyl-4-yl-5-oxo-pyrrolidin-1-yloxy)-4-oxo-3-(2-propenyl-penta-2,4-dienyloxycarbonyl amino)-pentanoic acid;
- 5-Benzoylaminooxy-3-benzyloxycarbonylamino-4-oxo-pentanoic acid;
- 3-Benzyloxycarbonylamino-4-oxo-5-(3-phenyl-propionyl-aminooxy)-pentanoic acid;
- 3-Benzyloxycarbonylamino-5-(2-naphthalen-1-yl-acetyl-aminooxy)-4-oxo-pentanoic acid;

- 3-Benzyloxycarbonylamino-5-(3-naphthalen-1-yl-propionylaminoxy)-4-oxo-pentanoic acid;
- 3-Benzyloxycarbonylamino-5-[methyl-(3-phenyl-propionyl)-aminoxy]-4-oxo-pentanoic acid;
- 5 5-(Benzoyl-methyl-aminoxy)-3-benzyloxycarbonylamino-4-oxo-pentanoic acid;
- 3-Benzyloxycarbonylamino-5-[methyl-(3-naphthalen-1-yl-propionyl)-aminoxy]-4-oxo-pentanoic acid;
- 3-Benzyloxycarbonylamino-5-[methyl-(naphthalen-1-yl-acetyl)-aminoxy]-4-oxo-pentanoic acid;
- 10 3-Benzyloxycarbonylamino-5-[benzyl-(3-phenyl-propionyl)-aminoxy]-4-oxo-pentanoic acid;
- 5-[Benzyl-(3-naphthalen-1-yl-propionyl)-aminoxy]-3-benzyloxycarbonylamino-4-oxo-pentanoic acid;
- 5-(3-Benzyl-2-oxo-pyrrolidin-1-yloxy)-4-oxo-3-(2-propenyl-penta-2,4-
- 15 dienyloxycarbonylamino)-pentanoic acid;
- 5-(3-Methyl-2-oxo-pyrrolidin-1-yloxy)-4-oxo-3-(2-propenyl-penta-2,4-dienyloxycarbonylamino)-pentanoic acid;
- 3-Benzyloxycarbonylamino-4-oxo-5-[methyl-(phenylacetyl)-aminoxy]-pentanoic acid; or
- 3-Benzyloxycarbonylamino-4-oxo-5-(1-oxo-1,3-dihydro-isoindol-2-yloxy)-pentanoic acid.
- 20 Hydroxamate ICE inhibitors as described above can be prepared, for example, as described in Example 12.

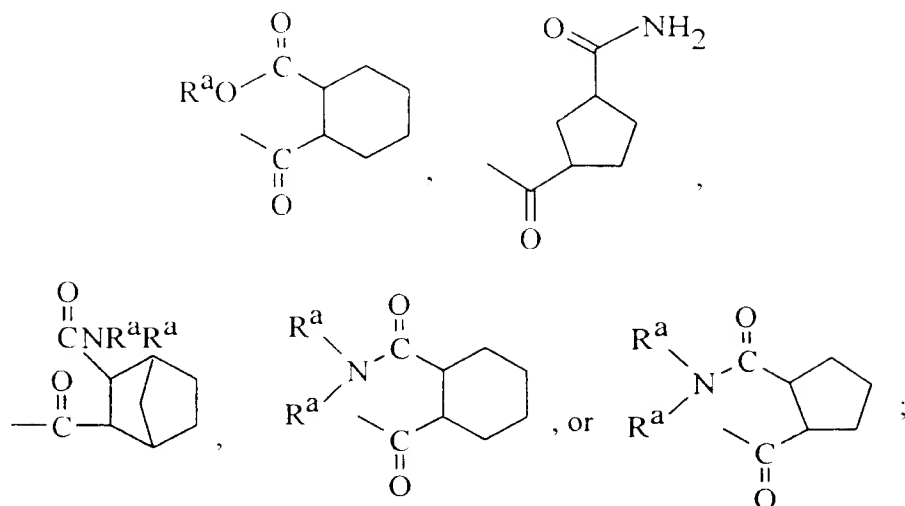
Still other types of ICE inhibitors that can be used in the invention include aspartate ester inhibitors, including compounds having the formula IX:



wherein R¹ is R³OC(O)-,
 R³CO-,
 R³SO₂-,
 R⁵N(R^a)CHR⁶CO-,

30

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each R^a is independently hydrogen, C_1 - C_6 alkyl, or $-(CH_2)_n$ aryl;

R^2 is $-(CRR)_n$ -aryl,

$-(CRR)_n$ -X-aryl,

5 $-(CRR)_n$ -heteroaryl,

$-(CRR)_n$ -X-heteroaryl,

$-(CRR)_n$ -(substituted-heteroaryl),

$-(CRR)_n$ -(substituted-aryl),

$-(CRR)_n$ -X-(substituted-aryl),

10 $-(CRR)_n$ -aryl-aryl,

$-(CRR)_n$ -aryl-heteroaryl,

$-(CRR)_n$ -aryl- $(CH_2)_n$ -aryl,

$-(CRR)_n$ -CH(aryl)₂,

$-(CRR)_n$ -cycloalkyl,

15 $-(CRR)_n$ -X-cycloalkyl,

$-(CRR)_n$ -heterocycle,

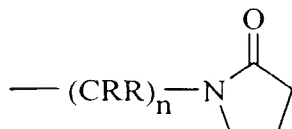
$-(CRR)_n$ -X-heterocycle,

$-(CRR)_n$ substituted heterocycle,

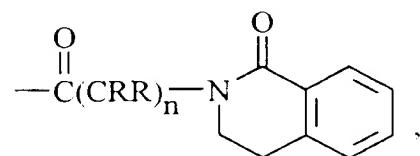
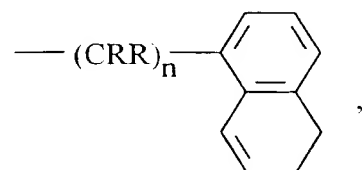
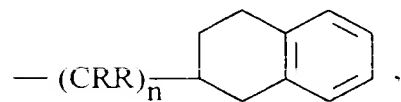
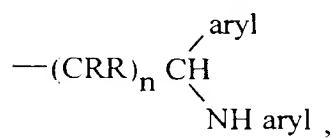
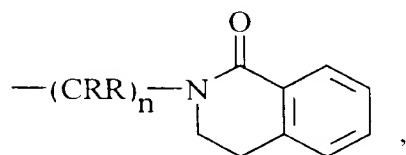
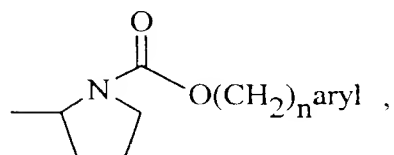
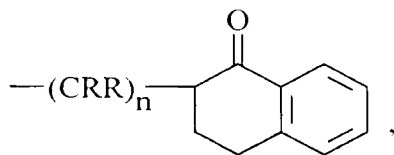
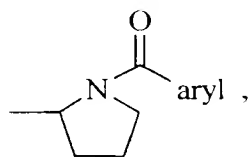
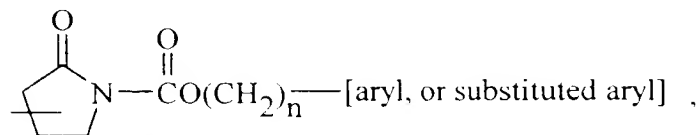
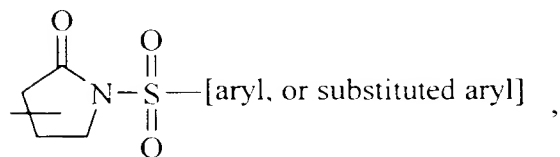
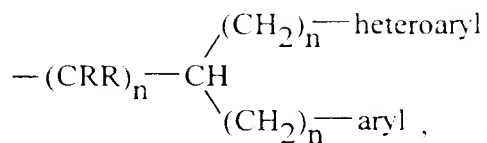
$-(CRR)_n$ -CH $\begin{matrix} \nearrow (CH_2)_n \text{ aryl} \\ \searrow (CH_2)_n \text{ aryl} \end{matrix}$,

$-(CRR)_n$ -CH $\begin{matrix} \nearrow (CH_2)_n \text{ substituted aryl} \\ \searrow (CH_2)_n \text{ aryl} \end{matrix}$,

20

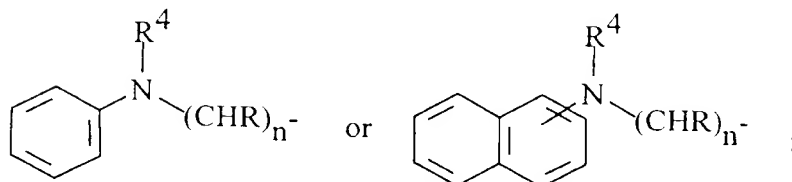
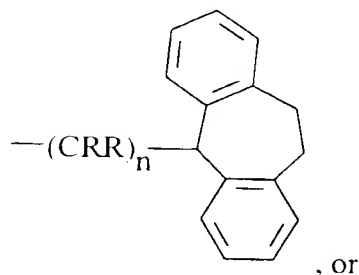


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5

10



each R is independently hydrogen, C₁-C₆ alkyl, halogen or hydroxy;

X is O or S;

R³ is C₁-C₆ alkyl,

aryl,

heteroaryl,

-(CHR)_n-aryl,

-(CHR)_n-heteroaryl,

-(CHR)_n-substituted heteroaryl,

-(CHR)_n-substituted aryl,

-(CRR)_nC(O)ORa,

-(CRR)_nS(CH₂)_n-aryl,

cycloalkyl,

substituted cycloalkyl,

heterocycle,

substituted heterocycle,

-(CRR)_nC(O)NRaRa

-(CRR)_n-SO₂-(CH₂)_n aryl,

-(CRR)_n-SO₂-C₁-C₆ alkyl,

J-CH₂C(R^a)H-,

-CHR⁶C(O)-heteroaryl,

-(CRR)_nS(CH₂)_nC(O)OR^a,

-(CRR)_n-SO₂-(CH₂)_nC(O)ORa,

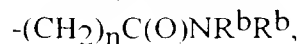
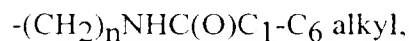
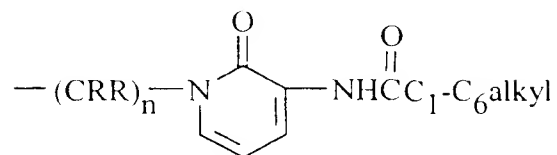
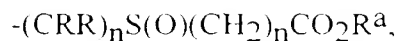
-(CRR)_nS(CH₂)_n-aryl,

-(CRR)_n-SO₂-(CH₂)_n-aryl,

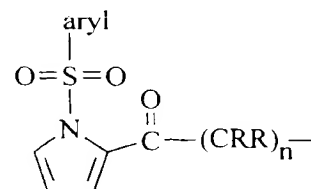
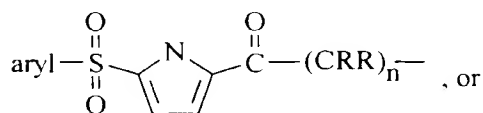
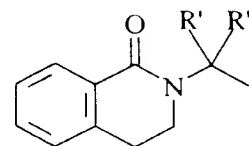
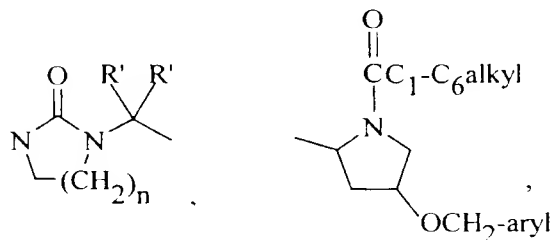
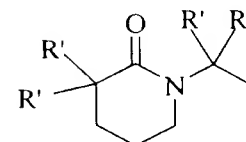
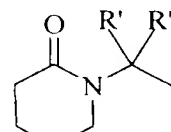
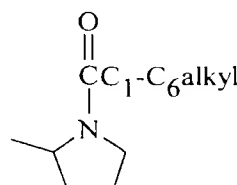
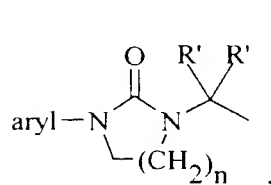
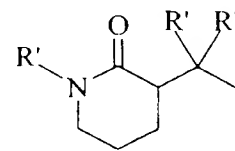
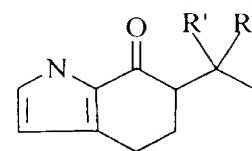
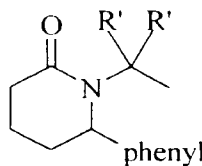
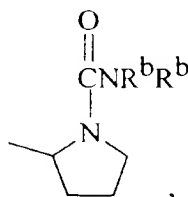
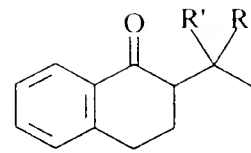
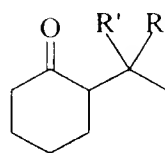
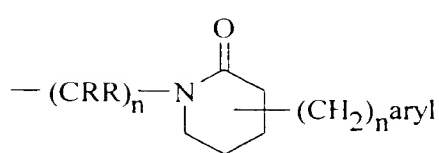
-(CRR)_nSC(O)C₁-C₆ alkyl,

-(CRR)_nS(O)(CH₂)_n aryl,

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5



each R' is independently C₁-C₆ alkyl,

C₁-C₆ alkylaryl,

aryl, or

hydrogen;

each J is independently

10

-CO₂R^b,
 -CONR^bR^b,
 -SO₂NR^bR^b, or
 -SO₂R^b;

5 each R^b is independently hydrogen, C₁-C₆ alkyl, aryl, substituted aryl; arylalkyl, heteroarylalkyl, substituted arylalkyl, or substituted heteroarylalkyl;

R⁴ is hydrogen,

C₁-C₆ alkyl,
 CH₃OC(O)-,
 10 -phenyl, or
 C₁-C₆ alkyl C(O)-;
 R⁵ is C₁-C₆ alkyl-CO-,
 -(CH₂)_n aryl,
 C₁-C₆-alkylOC(O)-,
 15 C₁-C₆-alkyl-X-(CH₂)_nCO,
 C₁-C₆-alkyl-X-(CH₂)_nOC(O)-,
 -C(O)(CRR)_naryl,
 -C(O)NR^aR^a,
 -SO₂-C₁-C₆ alkyl,
 20 -C(O)(CH₂)_nC(O)NR^aR^a,
 -C(O)O(CH₂)_n aryl,
 -C(O)O(CH₂)_n substituted aryl,
 -C(O)(CRR)_nNHC(O)O(CH₂)_n-aryl,

$$\begin{array}{c} \text{O} \\ \parallel \\ -\text{C}-\text{CH}-\text{N} \begin{array}{l} \nearrow \text{R}^a \\ \searrow \text{R}^{5a} \end{array} \\ | \\ \text{R}^6 \end{array}$$

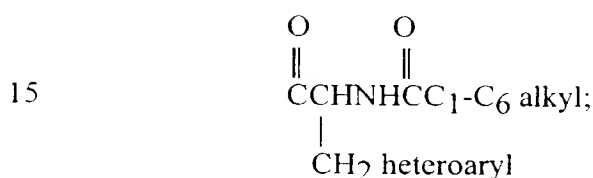
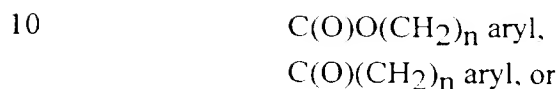
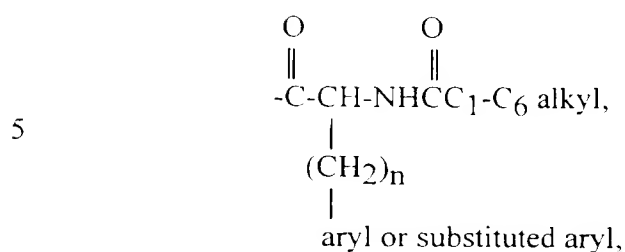
25 -(CH₂)_nX(CH₂)_n-aryl,
 -C₁-C₆ alkyl X-C₁-C₆ alkyl aryl, or

30
$$\begin{array}{c} \text{O} \quad \quad \text{O} \quad \quad \text{O} \\ \parallel \quad \quad \parallel \quad \quad \parallel \\ -\text{C}-\text{CH}-\text{NHC}-\text{CH}-\text{NHCC1-C6alkyl;} \\ | \quad \quad | \\ (\text{CH}_2)_n \quad \text{CH}_2\text{-heteroaryl} \\ | \\ \text{CO}_2\text{R}^a \end{array}$$

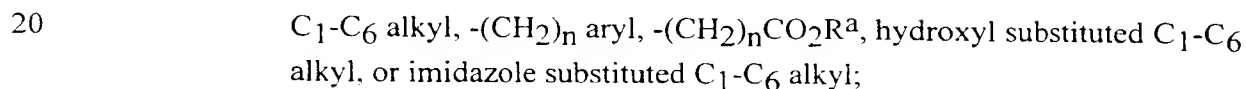
35

R^{5a} is

C(O)C₁-C₆ alkyl,
 -C(O)OC₁-C₆ alkyl,

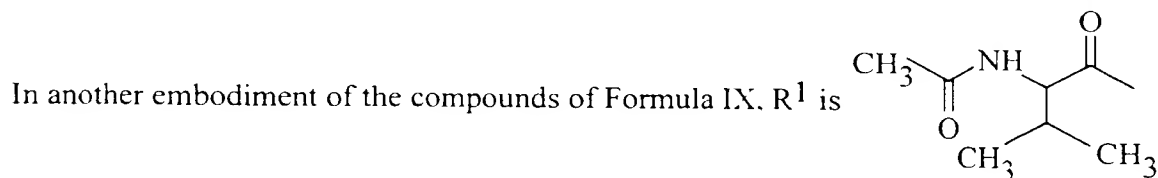
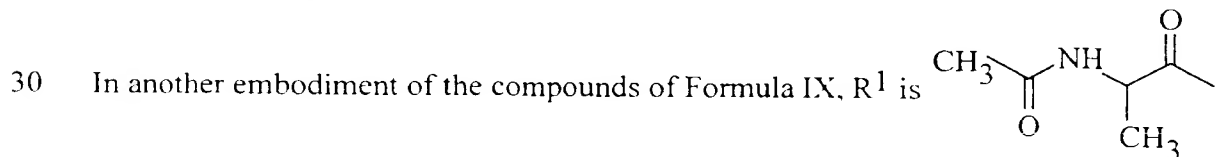


R^6 is hydrogen,

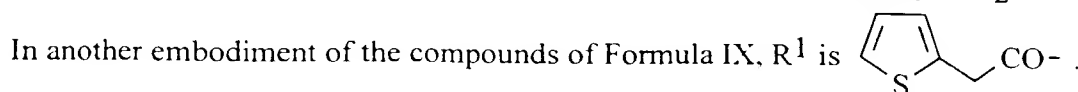


each n is independently 0 to 3, and the pharmaceutically acceptable, salts, esters, amides, and prodrugs thereof.

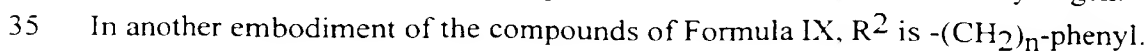
25 In one embodiment of the compounds of Formula IX, R^1 is phenyl- $\text{CH}_2\text{-OC(O)-}$.
 In another embodiment of the compounds of Formula IX, R^1 is phenyl- $\text{SO}_2\text{-}$.
 In another embodiment of the compounds of Formula IX, R^1 is $\text{CH}_3\text{-OC(O)-}$.
 In another embodiment of the compounds of Formula IX, R^1 is phenyl- $\text{CH}_2\text{CH}_2\text{-CO-}$.



In another embodiment of the compounds of Formula IX, R^1 is phenyl- $\text{CH}_2\text{-CO-}$.



In another embodiment of the compounds of Formula IX, each R^a is hydrogen.



In another embodiment of the compounds of Formula IX, R^2 is $-(CH_2)_n$ -naphthyl.

In another embodiment of the compounds of Formula IX, R^2 is $-(CH_2)_n$ -O-phenyl.

In another embodiment of the compounds of Formula IX, R^2 is $-(CH_2)_n$ -O-naphthyl.

In another embodiment of the compounds of Formula IX, R^2 is $-(CH_2)_n$ -S-phenyl.

- 5 In another embodiment of the compounds of Formula IX, R^2 is $-(CH_2)_n$ -CH(phenyl)₂.

In another embodiment of the compounds of Formula IX, each R^a is hydrogen; R^1 is benzyloxycarbonyl; R^2 is aryl-X(CRR)_n-, aryl-(CRR)_n-, heteroaryl-(CRR)_n-, or cycloalkyl-(CRR)_n-; n is 1, 2, or 3; X is O or S; and R is hydrogen, methyl, or benzyl.

- 10 In another embodiment of the compounds of Formula IX, each R^a is hydrogen;

R^1 is benzyloxycarbonyl; and

R^2 is $-(CH_2)_n$ -naphthyl,

$-(CH_2)_n$ -phenyl,

$-(CH_2)_n$ -cycloalkyl,

- 15 $-(CH_2)_n$ O(CH₂)_n-naphthyl,

$-(CH_2)_n$ O(CH₂)_n-phenyl, or

$-(CH_2)_n$ S(CH₂)_n-phenyl.

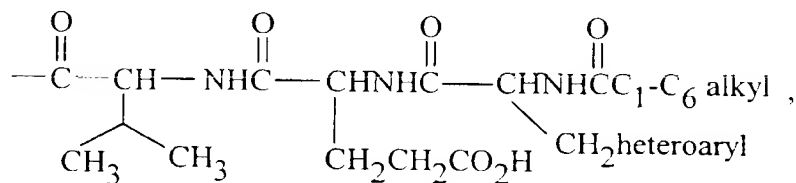
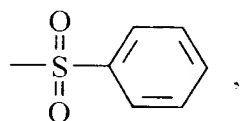
In another embodiment of the compounds of Formula IX, each R^a is hydrogen;

R^1 is benzyloxycarbonyl; and

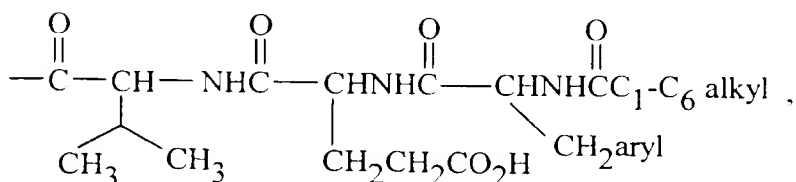
- 20 R^2 is $-CH_2$ -naphthyl.

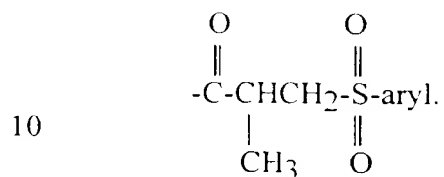
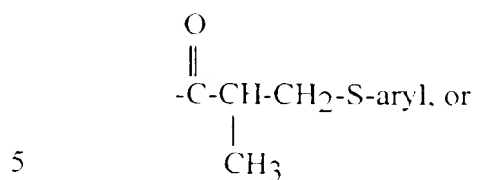
In another embodiment of the compounds of Formula IX, each R^a is hydrogen;

R^2 is benzyloxycarbonyl,



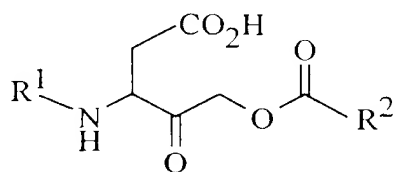
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Other aspartate ester ICE inhibitor compounds that can be used in the invention include compounds of the Formula X:

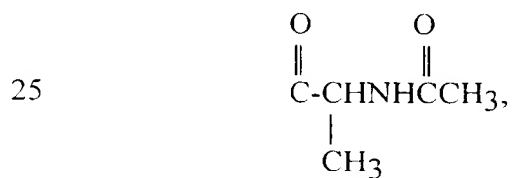
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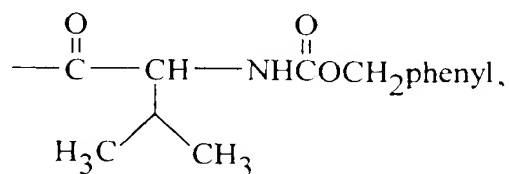
X

wherein R¹ is

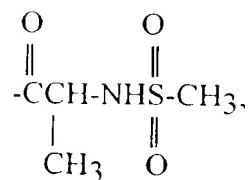
- 20
- C(O)OCH₂ phenyl,
 - SO₂-phenyl,
 - C(O)OCH₃,
 - C(O)CH₂CH₂ phenyl,



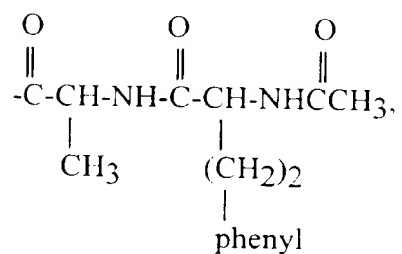
- 30
- C(O)CH₂ thienyl,
 - C(O)C₁-C₆ alkyl,
 - (CH₂)₃ phenyl,



5

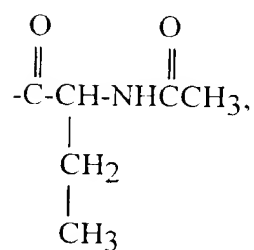


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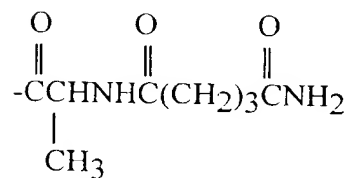


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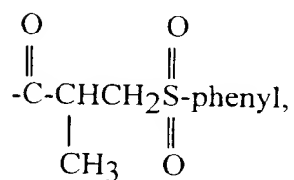
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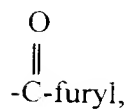
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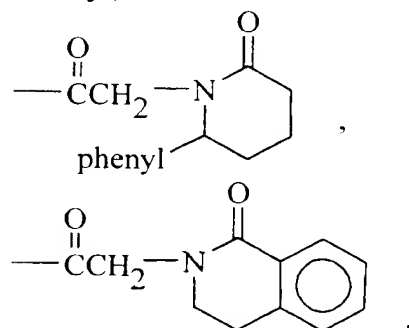
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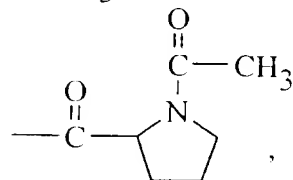
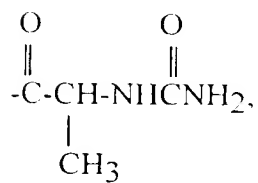
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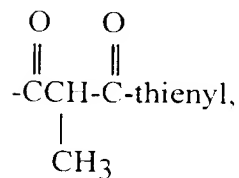
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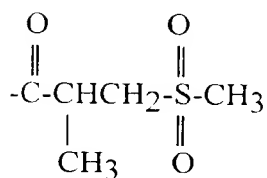
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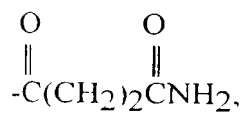
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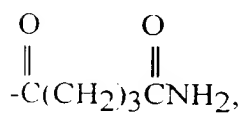
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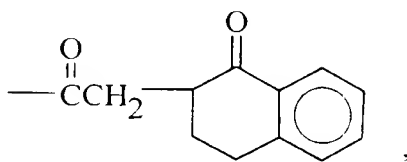
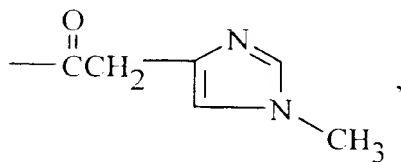
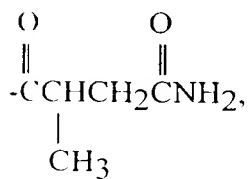
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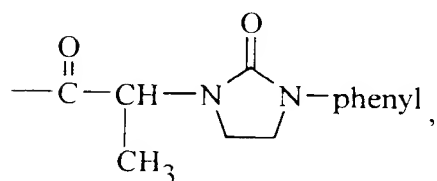
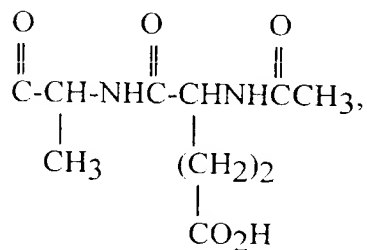
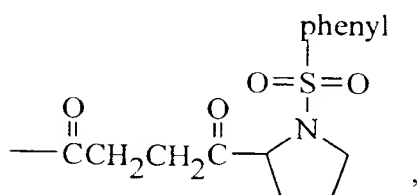
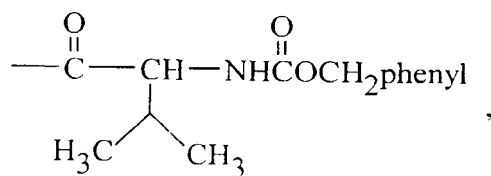
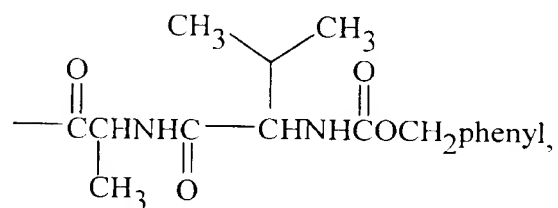
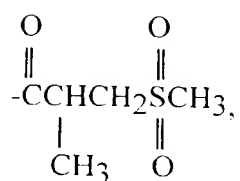
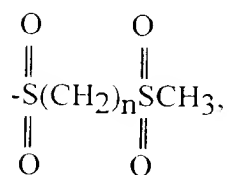
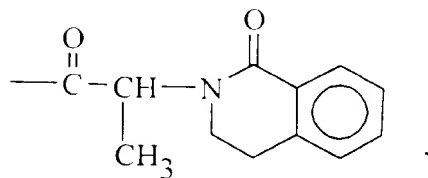


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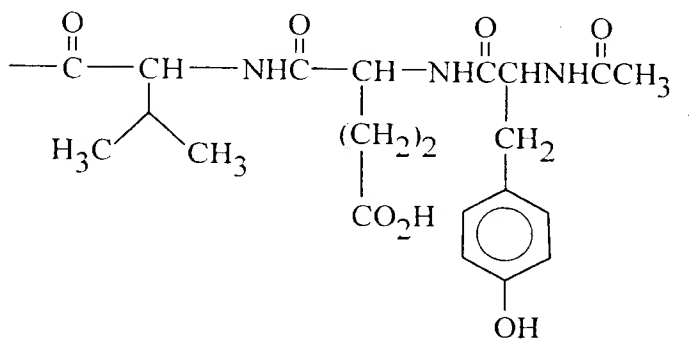
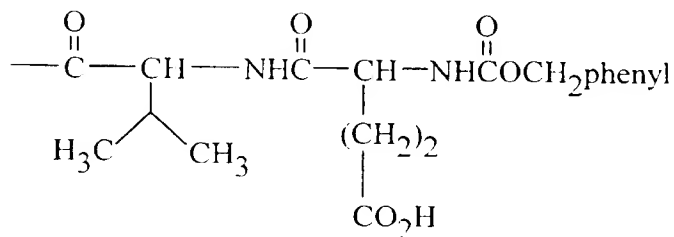
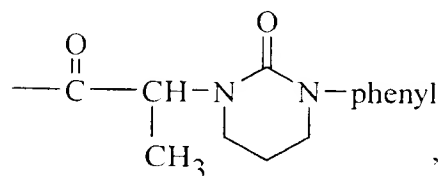
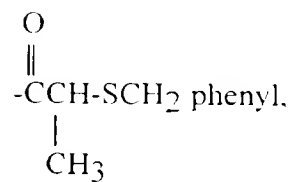


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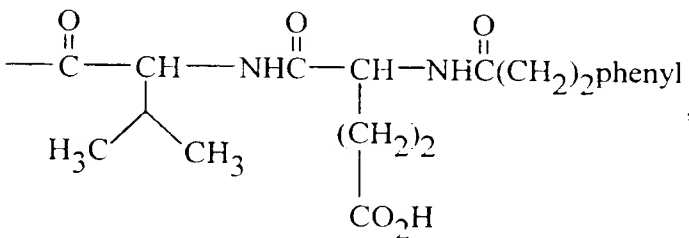
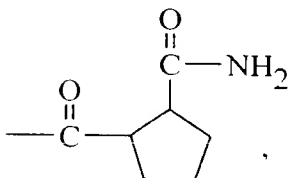
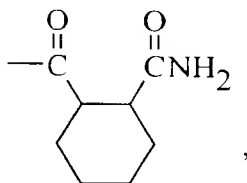




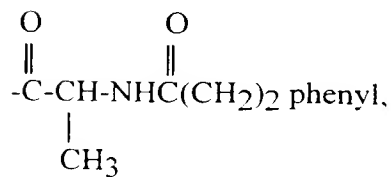
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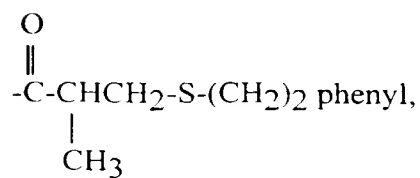
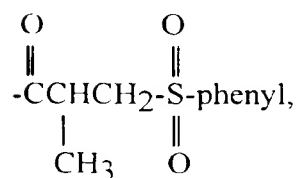
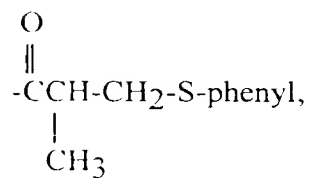
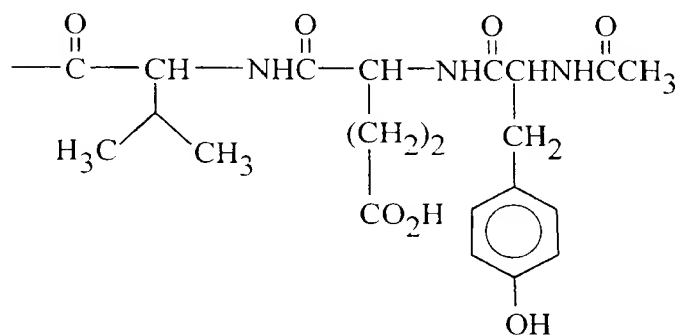
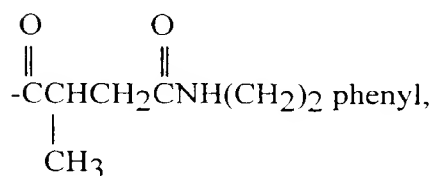
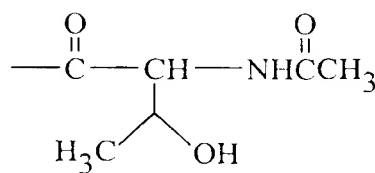
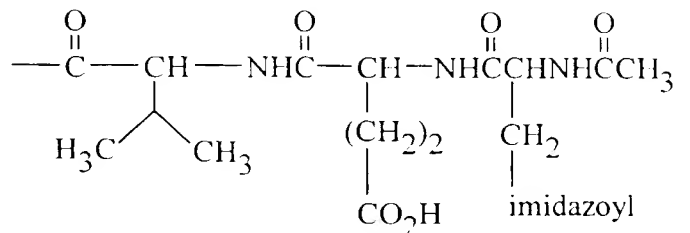
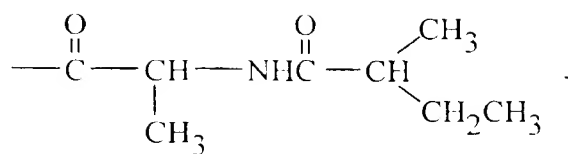


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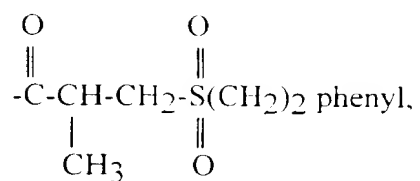


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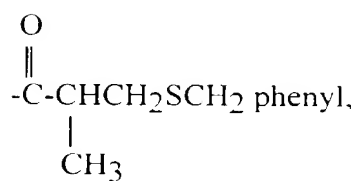




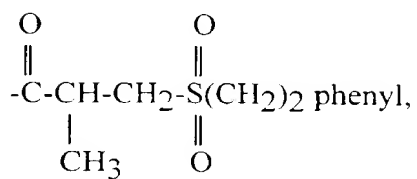
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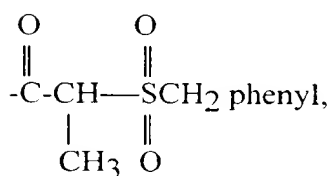
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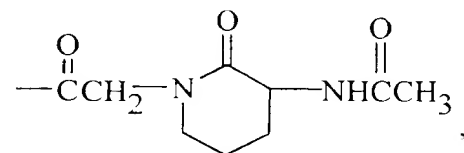
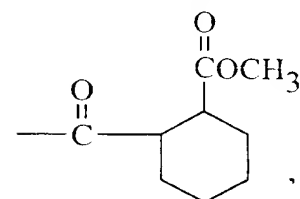
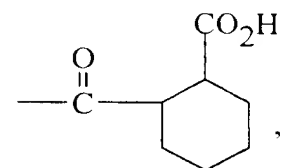
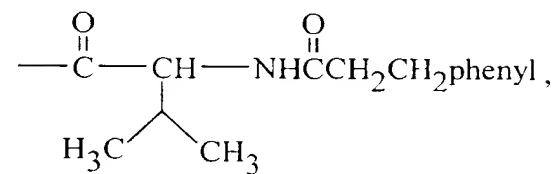
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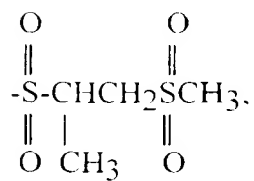


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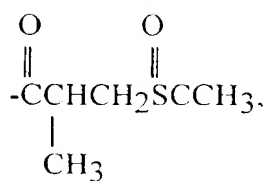


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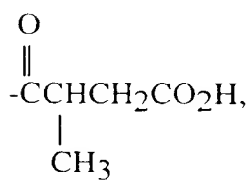
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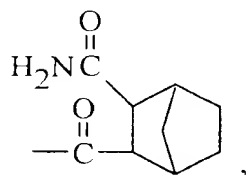
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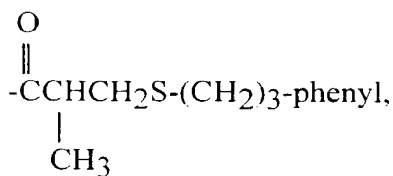
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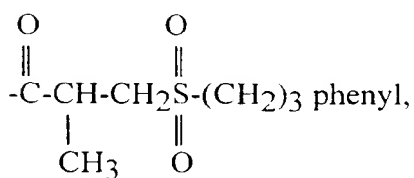
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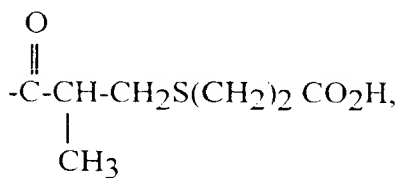
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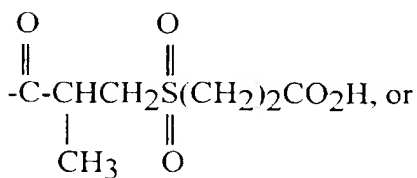
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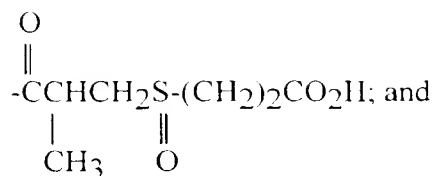
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5

R² is

- CH₂CH₂ phenyl,
 -CH₂ naphthyl,
 10 -CH₂CH₂ cyclohexyl,
 -CH₂O naphthyl,
 -CH₂O phenyl,
 -CH₂S-phenyl,
 -CH₂-substituted naphthyl,
 15 -CH₂CH(phenyl)₂,
 -CH₂-imidazole,
 -(CH₂)₃-phenyl,
 -C(CH₃)H-naphthyl,

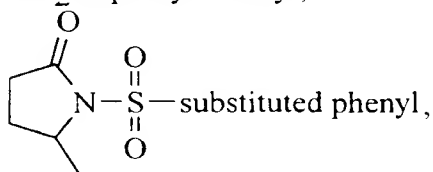
$$\begin{array}{c} \text{O} \\ \parallel \\ -\text{CH}_2-\text{N} \end{array} \text{Cyclopentyl},$$

$$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{C}=\text{O} \\ | \\ -\text{CH}_2-\text{N}-\text{phenyl} \end{array},$$

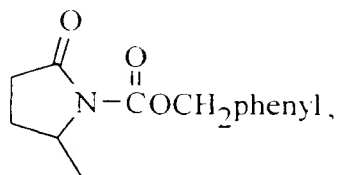
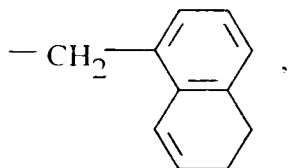
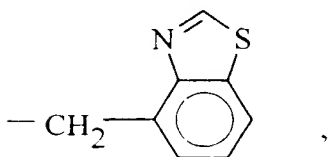
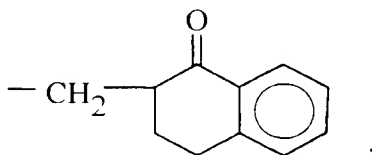
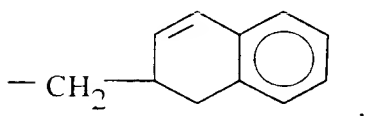
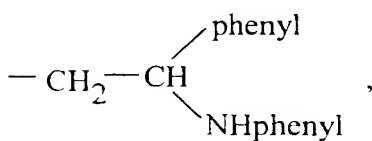
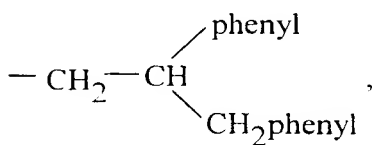
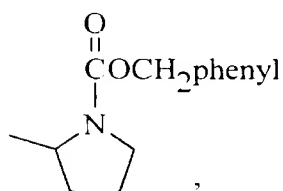
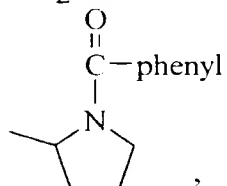
 20 -CH[CH₂phenyl]₂,
 -C(OH)H-naphthyl,
 -CH₂-NH phenyl,

$$\begin{array}{c} \text{CH}_2\text{substituted phenyl} \\ \diagup \\ -\text{CH}_2-\text{CH} \\ \diagdown \\ \text{naphthyl} \end{array},$$

 25 -CH₂-naphthyl-phenyl,
 -CH₂-fluorenyl,
 -CH₂ naphthyl-thienyl,

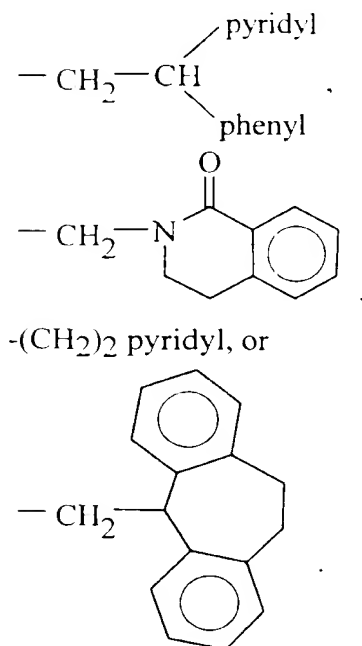


-44-

-CH₂-benzofuranyl,-CH₂-benzothieryl,-CH₂-naphthyl-CH₂ phenyl,-CH₂-substituted phenyl,-CH₂-substituted indolyl,

5

10



5 each n is independently 0 to 3, and the pharmaceutically acceptable, salts, esters, amides, and prodrugs thereof.

Preferred aspartate ester ICE inhibitor compounds include the compounds:

- 3-Benzylloxycarbonylamino-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;
- 10 3-Benzylloxycarbonylamino-4-oxo-5-(3-phenyl-propionyloxy)-pentanoic acid;
- 3-Benzylloxycarbonylamino-5-(3-cyclohexyl-propionyloxy)-4-oxo-pentanoic acid;
- 15 3-Benzylloxycarbonylamino-5-[(naphthalene-1-yl-oxy)-acetoxy]-4-oxo-pentanoic acid;
- 3-Benzylloxycarbonylamino-4-oxo-5-phenoxyacetoxy-pentanoic acid;
- 3-Benzylloxycarbonylamino-4-oxo-5-phenylsulfanylacetoxy-pentanoic acid;
- 3-Benzylloxycarbonylamino-5-[(6-methoxy-naphthalene-1-yl)-acetoxy]-4-oxo-pentanoic acid;
- 20 3-Benzylloxycarbonylamino-5-(naphthalene-2-yl-acetoxy)-4-oxo-pentanoic acid;
- 3-Benzylloxycarbonylamino-5-(3-naphthalene-2-yl-propionyloxy)-4-oxo-pentanoic acid;
- 3-Benzylloxycarbonylamino-5-(3,3-diphenyl-propionyloxy)-4-oxo-pentanoic acid;
- 25 3-Benzylloxycarbonylamino-5-[(1H-indol-3-yl)-acetoxy]-4-oxo-pentanoic acid;
- 3-Benzylloxycarbonylamino-5-(indol-1-yl-acetoxy)-4-oxo-pentanoic acid;

- 3-Benzylloxycarbonylamino-5-(2-naphthalene-1-yl-propionyloxy)-4-oxo-pentanoic acid;
- 3-Benzylloxycarbonylamino-4-oxo-5-[(2-oxo-pyrrolidin-1-yl)-acetoxy]-pentanoic acid;
- 5 3-[(Acetyl-phenyl-amino)-acetoxy]-3-benzylloxycarbonyl-amino-4-oxo-pentanoic acid;
- 3-Benzylloxycarbonylamino-5-(2-benzyl-3-phenyl-propionyloxy)-4-oxo-pentanoic acid;
- 3-Benzylloxycarbonylamino-5-(hydroxy-naphthalene-1-yl-acetoxy)-4-oxo-pentanoic acid;
- 10 3-Benzylloxycarbonylamino-4-oxo-5-[(phenyl-amino)-acetoxy]-pentanoic acid;
- 3-Benzylloxycarbonylamino-5-[(6-hydroxy-naphthalene-1-yl)-acetoxy]-4-oxo-pentanoic acid;
- 15 3-Benzylloxycarbonylamino-5-[3-(4-hydroxy-phenyl)-2-naphthalene-1-yl-propionyloxy]-4-oxo-pentanoic acid;
- (S)-3-Benzylloxycarbonylamino-4-oxo-5-phenylacetoxy-pentanoic acid;
- (S)-3-Benzylloxycarbonylamino-4-oxo-5-(4-phenyl-butyryloxy)-pentanoic acid;
- 20 3-Benzylloxycarbonylamino-4-oxo-5-[(4-phenyl-naphthalen-1-yl)-acetoxy]-pentanoic acid;
- 3-Benzylloxycarbonylamino-5-[(4-methyl-naphthalen-1-yl)-acetoxy]-4-oxo-pentanoic acid;
- 3-Benzylloxycarbonylamino-4-oxo-5-[(4-thiophen-2-yl-naphthalen-1-yl)-acetoxy]-pentanoic acid;
- 25 3-Benzylloxycarbonylamino-5-[(4-fluoro-naphthalen-1-yl)-acetoxy]-4-oxo-pentanoic acid;
- 3-Benzylloxycarbonylamino-5-[(2-methyl-naphthalen-1-yl)-acetoxy]-4-oxo-pentanoic acid;
- 30 3-Benzylloxycarbonylamino-5-[(2-fluoro-naphthalen-1-yl)-acetoxy]-4-oxo-pentanoic acid;
- 5-(Benzofuran-4-yl-acetoxy)-3-benzylloxycarbonylamino-4-oxo-pentanoic acid;
- 5-(Benzo[b]thiophen-7-yl-acetoxy)-3-benzylloxycarbonylamino-4-oxo-pentanoic acid;
- 35 5-(Benzo[b]thiophen-4-yl-acetoxy)-3-benzylloxycarbonylamino-4-oxo-pentanoic acid;

5-[(4-Benzyl-naphthalen-1-yl)-acetoxy]-3-benzyloxycarbonylamino-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-[(3,4-dihydro-naphthalen-1-yl)-acetoxy]-4-oxo-pentanoic acid;

5 3-Benzyloxycarbonylamino-5-[(5-bromo-1H-indol-3-yl)-acetoxy]-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-(3,4-diphenyl-butyryloxy)-4-oxo-pentanoic acid;

10 3-Benzyloxycarbonylamino-4-oxo-5-(3-phenyl-3-phenylamino-propionyloxy)-pentanoic acid;

3-Benzyloxycarbonylamino-4-oxo-5-[(1,2,3,4-tetrahydro-naphthalen-2-yl)-acetoxy]-pentanoic acid;

3-Benzyloxycarbonylamino-5-[(1-methanesulfonyl-piperidin-4-yl)-acetoxy]-4-oxo-pentanoic acid;

15 3-Benzyloxycarbonylamino-4-oxo-5-[(2,3,5,6-tetramethyl-phenyl)-acetoxy]-pentanoic acid;

5-(Benzothiazol-4-yl-acetoxy)-3-benzyloxycarbonylamino-4-oxo-pentanoic acid;

20 5-(Benzofuran-3-yl-acetoxy)-3-benzyloxycarbonylamino-4-oxo-pentanoic acid;

5-(Benzo[b]thiophen-3-yl-acetoxy)-3-benzyloxycarbonylamino-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-4-oxo-5-(3-phenyl-3-pyridin-2-yl-propionyloxy)-pentanoic acid;

25 3-Benzyloxycarbonylamino-5-[(2,3-dichloro-phenyl)-acetoxy]-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-[(5-methyl-naphthalen-1-yl)-acetoxy]-4-oxo-pentanoic acid;

30 3-Benzyloxycarbonylamino-5-[(2-iodo-phenyl)-acetoxy]-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-4-oxo-5-(3-pyridin-3-yl-propionyloxy)-pentanoic acid;

3-Benzyloxycarbonylamino-5-[(5-methoxy-naphthalen-1-yl)-acetoxy]-4-oxo-pentanoic acid;

35 3-Benzyloxycarbonylamino-5-[(8-methyl-naphthalen-1-yl)-acetoxy]-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-[(9H-fluoren-9-yl)-acetoxy]-4-oxo-pentanoic acid;

- 3-Benzyloxycarbonylamino-5-[(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-yl)-acetoxy]-4-oxo-pentanoic acid;
- 5-Oxo-1-(toluene-4-sulfonyl)-pyrrolidine-2-carboxylic acid
- 3-benzyloxycarbonylamino-4-carboxy-2-oxo-butyl ester;
- 5-Oxo-pyrrolidine-1,2-dicarboxylic acid 1-benzyl ester 2-(3-benzyloxycarbonylamino-4-carboxy-2-oxo-butyl) ester;
- 1-Benzoyl-pyrrolidine-2-carboxylic acid 3-benzyloxycarbonylamino-4-carboxy-2-oxo-butyl ester;
- Pyrrolidine-1,2-dicarboxylic acid 1-benzyl ester 2-(3-benzyloxycarbonylamino-4-carboxy-2-oxo-butyl) ester;
- 3-Benzyloxycarbonylamino-5-(2-benzyl-3-phenyl-propionyloxy)-4-oxo-pentanoic acid;
- 3-Benzyloxycarbonylamino-5-[(5-cyano-naphthalen-1-yl)-acetoxy]-4-oxo-pentanoic acid;
- 3-Benzyloxycarbonylamino-4-oxo-5-(3-phenyl-3-pyridin-3-yl-propionyloxy)-pentanoic acid;
- 3-Benzyloxycarbonylamino-4-oxo-5-(3-phenyl-3-pyridin-4-yl-propionyloxy)-pentanoic acid; and
- 3-Benzyloxycarbonylamino-4-oxo-5-[(1-oxo-3,4-dihydro-1H-isoquinolin-2-yl)-acetoxy]-pentanoic acid.
- 3-Benzenesulfonylamino-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;
- 3-Methoxycarbonylamino-5-(naphthalene-1-yl-acetoxy)-4-oxo-pentanoic acid;
- 5-(Naphthalene-1-yl-acetoxy)-4-oxo-3-(3-phenyl-propionylamino)-pentanoic acid;
- 3-Methoxycarbonylamino-4-oxo-5-phenoxyacetoxy-pentanoic acid; and
- 3-(2-Methanesulfonyl-1-methyl-ethylsulfanylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid.
- [S-(R*,R*)]-3-(2-Acetyl-amino-propionylamino)-5-(naphthalene-1-yl-acetoxy)-4-oxo-pentanoic acid;
- 5-(Naphthalen-1-yl-acetoxy)-4-oxo-3-[(thiophene-3-carbonyl)-amino]-pentanoic acid;
- 3-[(Furan-3-carbonyl)-amino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;
- 5-(Naphthalen-1-yl-acetoxy)-4-oxo-3-[2-(4-phenyl-butyrylamino)-propylamino]-pentanoic acid;
- 3-(2-Methanesulfonylamino-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-[2-(2-Acetylamino-4-phenyl-butyrylamino)-propionylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(2-Acetylamino-butyrylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

5 3-[2-(4-Carbamoyl-butyrylamino)-propionylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(2-Benzyloxycarbonylamino-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

10 5-(Naphthalen-1-yl-acetoxy)-4-oxo-3-(2-ureido-propionylamino)-pentanoic acid;

3-(2-Acetylamino-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-[(1-Acetyl-pyrrolidine-2-carbonyl)-amino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

15 3-(2-Methyl-3-oxo-3-thiophen-2-yl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(2-Acetylamino-acetylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

20 3-(2-Acetylamino-propionylamino)-5-(3,3-diphenyl-propionyloxy)-4-oxo-pentanoic acid;

3-[2-(2-Acetylamino-4-carboxy-butyrylamino)-propionylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

5-(Naphthalen-1-yl-acetoxy)-4-oxo-3-[2-(3-phenyl-propionylamino)-propionylamino]-pentanoic acid;

25 3-[2-(3-Methyl-butyrylamino)-propionylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-[(1-Acetyl-4-benzyloxy-pyrrolidine-2-carbonyl)-amino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

30 3-(4-Carbamoyl-butyrylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid; and

3-[2-(1-Methyl-1H-imidazol-4-yl)-acetylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid.

(S)-5-(Naphthalene-1-yl-acetoxy)-4-oxo-3-phenylacetylamino-pentanoic acid;

35 (S)-5-(Naphthalene-1-yl-acetoxy)-4-oxo-3-(2-thiophene-2-yl-acetylamino)-pentanoic acid;

3-[(2-Carbamoyl-cyclopentanecarbonyl)-amino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-[(3-Carbamoyl-bicyclo[2.2.1]heptane-2-carbonyl)-amino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(3-Methanesulfonyl-2-methyl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

5 3-(3-Benzenesulfonyl-2-methyl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-Butyrylamino-5-(naphthalen-2-yl-acetoxy)-4-oxo-pentanoic acid;

3-Acetylamino-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

10 3-(3-Methanesulfonyl-2-methyl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(3-Methyl-butyrylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(3-Carbamoyl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

15 [S-(R*,R*)]-3-(3-Acetylsulfonyl-2-methyl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid; and

trans-3-[(3-Carbamoyl-cyclopentanecarbonyl)-amino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid.

20 3-(1,2,3,4-tetrahydro-1-oxo-isoquinoline-2-yl)-acetamino-5-(naphthalene-1-yl acetoxyl)-4-oxo-pentanoic acid;

3-(2-Methyl-3-phenethylcarbamoyl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

5-(Naphthalen-2-yl-acetoxy)-4-oxo-3-[2-(2-oxo-6-phenyl-piperidin-1-yl)-acetylamino]-pentanoic acid;

25 5-(Naphthalen-1-yl-acetoxy)-4-oxo-3-[2-(2-oxo-6-phenyl-piperidin-1-yl)-acetylamino]-pentanoic acid;

3-[3-Methyl-2-(3-phenyl-propionylamino)-butyrylamino]-4-oxo-5-[(1-oxo-1,2,3,4-tetrahydro-naphthalen-2-yl)-acetoxy]-pentanoic acid;

30 5-(Naphthalen-2-yl-acetoxy)-4-oxo-3-[2-(1-oxo-3,4-dihydro-1H-isoquinolin-2-yl)-acetylamino]-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-4-oxo-3-[2-(1-oxo-3,4-dihydro-1H-isoquinolin-2-yl)-acetylamino]-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-4-oxo-3-[2-(2-oxo-6-phenyl-piperidin-1-yl)-acetylamino]-pentanoic acid;

35 5-(Naphthalen-1-yl-acetoxy)-4-oxo-3-[2-(1-oxo-1,2,3,4-tetrahydro-naphthalen-2-yl)-acetylamino]-pentanoic acid;

5-(Naphthalen-1-yl-acetoxy)-4-oxo-3-[2-(1-oxo-3,4-dihydro-1H-isoquinolin-2-yl)-propionylamino]-pentanoic acid;

5-(Naphthalen-2-yl-acetoxy)-4-oxo-3-[2-(1-oxo-3,4-dihydro-1H-isoquinolin-2-yl)-propionylamino]-pentanoic acid;

3-[4-(1-Benzenesulfonyl-1H-pyrrol-2-yl)-4-oxo-butyrylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

5 5-(2-Benzyl-3-phenyl-propionyloxy)-4-oxo-3-[2-(1-oxo-1,2,3,4-tetrahydro-naphthalen-2-yl)-acetylamino]-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-4-oxo-3-[2-(1-oxo-3,4-dihydro-1H-isoquinolin-2-yl)-propionylamino]-pentanoic acid;

10 4-Oxo-3-[2-(1-oxo-3,4-dihydro-1H-isoquinolin-2-yl)-propionylamino]-5-[(1-oxo-1,2,3,4-tetrahydro-naphthalen-2-yl)-acetoxy]-pentanoic acid;

3-[4-(1-Benzenesulfonyl-1H-pyrrol-2-yl)-4-oxo-butyrylamino]-5-(2-benzyl-3-phenyl-propionyloxy)-4-oxo-pentanoic acid;

4-Oxo-5-[(1-oxo-1,2,3,4-tetrahydro-naphthalen-2-yl)-acetoxy]-3-[2-(1-oxo-1,2,3,4-tetrahydro-naphthalen-2-yl)-acetylamino]-pentanoic acid;

15 5-(Naphthalen-1-yl-acetoxy)-4-oxo-3-[2-(2-oxo-3-phenyl-imidazolidin-1-yl)-propionylamino]-pentanoic acid;

5-(Naphthalen-1-yl-acetoxy)-4-oxo-3-[2-(2-oxo-3-phenyl-tetrahydro-pyrimidin-1-yl)-propionylamino]-pentanoic acid;

20 5-(Naphthalen-1-yl-acetoxy)-4-oxo-3-[2-(2-oxo-3-phenyl-tetrahydro-pyrimidin-1-yl)-acetylamino]-pentanoic acid;

3-(2-Acetyl-amino-3-methyl-butyrylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(2-Acetyl-amino-3-methyl-butyrylamino)-5-(2-benzyl-3-phenyl-propionyloxy)-4-oxo-pentanoic acid;

25 3-(2-Acetyl-amino-3-methyl-butyrylamino)-5-(3-benzyl-4-phenyl-butyryloxy)-4-oxo-pentanoic acid;

3-(2-Acetyl-amino-3-methyl-butyrylamino)-5-(4-benzyl-5-phenyl-pentanoyloxy)-4-oxo-pentanoic acid;

30 3-(2-Acetyl-amino-3-methyl-butyrylamino)-4-oxo-5-[(1-oxo-1,2,3,4-tetrahydro-naphthalen-2-yl)-acetoxy]-pentanoic acid;

5-(3-Benzyl-4-phenyl-butyryloxy)-3-[3-methyl-2-(3-phenyl-propionylamino)-butyrylamino]-4-oxo-pentanoic acid;

3-[2-(3-Acetyl-amino-2-oxo-2H-pyridin-1-yl)-acetylamino]-5-(3,3-diphenyl-propionyloxy)-4-oxo-pentanoic acid; and

35 3-[2-(3-Acetyl-amino-2-oxo-2H-pyridin-1-yl)-acetylamino]-5-(2-benzyl-3-phenyl-propionyloxy)-4-oxo-pentanoic acid.

3-[2-(2-Benzylloxycarbonylamino-4-carboxy-butyrylamino)-3-methyl-butyrylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-[2-(2-Benzoyloxycarbonylamino-3-methyl-butyrylamino)-
propionylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(2-Acetylamino-3-methyl-butyrylamino)-5-(naphthalen-1-yl-acetoxy)-
4-oxo-pentanoic acid;

5 3-[2-(2-Benzoyloxycarbonylamino-3-methyl-butyrylamino)-
propionylamino]-5-(3,3-diphenyl-propionyloxy)-4-oxo-pentanoic acid;

3-[2-(2-Benzoyloxycarbonylamino-3-methyl-butyrylamino)-
propionylamino]-5-(2-benzyl-3-phenyl-propionyloxy)-4-oxo-pentanoic acid;

10 3-[2-(2-Benzoyloxycarbonylamino-3-methyl-butyrylamino)-
propionylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-3-{2-[4-carboxy-2-(3-phenyl-
propionylamino)-butyrylamino]-3-methyl-butyrylamino}-4-oxo-pentanoic acid;

3-(2-Benzoyloxycarbonylamino-3-methyl-butyrylamino)-5-(3,3-diphenyl-
propionyloxy)-4-oxo-pentanoic acid;

15 3-(2-Acetylamino-3-hydroxy-butyrylamino)-5-(naphthalen-1-yl-acetoxy)-
4-oxo-pentanoic acid;

3-(2-Acetylamino-3-hydroxy-butyrylamino)-5-(3,3-diphenyl-propionyloxy)-
4-oxo-pentanoic acid;

20 3-(2-{2-[2-Acetylamino-3-(1H-indol-3-yl)-propionylamino]-4-carboxy-
butyrylamino}-3-methyl-butyrylamino)-5-(2-benzyl-3-phenyl-propionyloxy)-4-oxo-
pentanoic acid; and

5-(3,3-Diphenyl-propionyloxy)-4-oxo-3-[2-(4-phenyl-butyrylamino)-
propionylamino]-pentanoic acid.

25 3-(2-{2-[2-Acetylamino-3-(1H-indol-3-yl)-propionylamino]-4-carboxy-
butyrylamino}-3-methyl-butyrylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-
pentanoic acid; and

3-(2-{2-[2-Acetylamino-3-(4-hydroxy-phenyl)-propionylamino]-4-carboxy-
butyrylamino}-3-methyl-butyrylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-
pentanoic acid.

30 3-[(2-Carboxy-cyclohexanecarbonyl)-amino]-5-(naphthalen-1-yl-acetoxy)-
4-oxo-pentanoic acid;

3-[(2-Methoxycarbonyl-cyclohexanecarbonyl)-amino]-5-(naphthalen-1-yl-
acetoxy)-4-oxo-pentanoic acid; and

35 3-[(2-Carbamoyl-cyclohexanecarbonyl)-amino]-5-(naphthalen-1-yl-
acetoxy)-4-oxo-pentanoic acid.

3-(3-Benzylsulfanyl-2-methyl-propionylamino)-5-(naphthalen-1-yl-
acetoxy)-4-oxo-pentanoic acid;

3-(2-Methyl-3-phenylmethanesulfonyl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-[3-(2-Carboxy-ethanesulfanyl)-2-methyl-propionylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

5 5-(2-Benzyl-3-phenyl-propionyloxy)-3-[3-(2-carboxy-ethanesulfonyl)-2-methyl-propionylamino]-4-oxo-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-3-[3-(3-carboxy-propane-1-sulfinyl)-2-methyl-propionylamino]-4-oxo-pentanoic acid;

10 5-(Naphthalen-1-yl-acetoxy)-4-oxo-3-(2-phenylmethanesulfanyl-propionylamino)-pentanoic acid;

3-(2-Methyl-3-phenylsulfanyl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-3-(2-methyl-3-phenylsulfanyl-propionylamino)-4-oxo-pentanoic acid;

15 3-(2-Methyl-3-phenethylsulfanyl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-3-(2-methyl-3-phenethylsulfanyl-propionylamino)-4-oxo-pentanoic acid;

20 5-(2-Benzyl-3-phenyl-propionyloxy)-3-(3-benzylsulfanyl-2-methyl-propionylamino)-4-oxo-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-3-(2-benzylsulfanyl-propionylamino)-4-oxo-pentanoic acid;

3-[2-Methyl-3-(3-phenyl-propylsulfanyl)-propionylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

25 3-(3-Benzenesulfonyl-2-methyl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(3-Benzenesulfonyl-2-methyl-propionylamino)-5-(2-benzyl-3-phenyl-propionyloxy)-4-oxo-pentanoic acid;

30 5-(2-Benzyl-3-phenyl-propionyloxy)-3-[2-methyl-3-(2-phenyl-ethanesulfonyl)-propionylamino]-4-oxo-pentanoic acid;

3-[2-Methyl-3-(2-phenyl-ethanesulfonyl)-propionylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

5-(Naphthalen-1-yl-acetoxy)-4-oxo-3-(2-phenylmethanesulfonyl-propionylamino)-pentanoic acid;

35 5-(2-Benzyl-3-phenyl-propionyloxy)-3-(2-methyl-3-phenylmethanesulfonyl-propionylamino)-4-oxo-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-4-oxo-3-(2-phenylmethanesulfonyl-propionylamino)-pentanoic acid;

3-[2-Methyl-3-(3-phenyl-propane-1-sulfonyl)-propionylamino]-
5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-3-[2-methyl-3-(3-phenyl-propane-
1-sulfonyl)-propionylamino]-4-oxo-pentanoic acid;

5 5-(2-Benzyl-3-phenyl-propionyloxy)-3-[3-(2-carboxy-ethylsulfanyl)-
2-methyl-propionylamino]-4-oxo-pentanoic acid;

3-[3-(3-Carboxy-propylsulfanyl)-2-methyl-propionylamino]-5-(naphthalen-
1-yl-acetoxy)-4-oxo-pentanoic acid;

10 5-(2-Benzyl-3-phenyl-propionyloxy)-3-[3-(3-carboxy-propylsulfanyl)-
2-methyl-propionylamino]-4-oxo-pentanoic acid;

3-(3-Carboxymethylsulfanyl-2-methyl-propionylamino)-5-(naphthalen-1-yl-
acetoxy)-4-oxo-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-3-(3-carboxymethylsulfanyl-2-methyl-
propionylamino)-4-oxo-pentanoic acid;

15 3-[3-(2-Carboxy-ethanesulfonyl)-2-methyl-propionylamino]-5-(naphthalen-
1-yl-acetoxy)-4-oxo-pentanoic acid;

3-[3-(3-Carboxy-propane-1-sulfonyl)-2-methyl-propionylamino]-
5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

20 3-(3-Carboxymethanesulfonyl-2-methyl-propionylamino)-5-(naphthalen-
1-yl-acetoxy)-4-oxo-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-3-[3-(3-carboxy-propane-1-sulfonyl)-
2-methyl-propionylamino]-4-oxo-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-3-(3-carboxymethanesulfonyl-
2-methyl-propionylamino)-4-oxo-pentanoic acid;

25 3-[3-(3-Carboxy-propane-1-sulfinyl)-2-methyl-propionylamino]-
5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-[2-Methyl-3-(3-phenyl-propane-1-sulfinyl)-propionylamino]-
5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid; and

30 5-(2-Benzyl-3-phenyl-propionyloxy)-3-[2-methyl-3-(3-phenyl-propane-
1-sulfinyl)-propionylamino]-4-oxo-pentanoic acid.

3-[3-Methyl-2-(phenethylcarbamoyl-methyl)-butyrylamino]-5-(naphthalen-
1-yl-acetoxy)-4-oxo-pentanoic acid; and

3-(3-Carboxy-2-methyl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-
oxo-pentanoic acid.

35 3-(2-Methyl-3-sulfamoyl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-
oxo-pentanoic acid.

3-(3-Carbamoyl-2-methyl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-
oxo-pentanoic acid;

3-(2-Benzyloxycarbonylamino-3-methyl-naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-[(2-Carbamoyl-cyclopentanecarbonyl)-amino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

5 3-[(1-Carbamoyl-pyrrolidine-2-carbonyl)-amino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(2-{2-[2-Acetylamino-3-(4-hydroxy-phenyl)-propionylamino]-4-carboxy-butyrylamino}-3-methyl-butyrylamino)-5-(2-benzyl-3-phenyl-propionyloxy)-4-oxo-pentanoic acid;

10 3-(3-Carbamoyl-2-methyl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(2-Carbamoylmethyl-3-methyl-butyrylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

15 3-(3-Benzyloxy-2-ureido-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

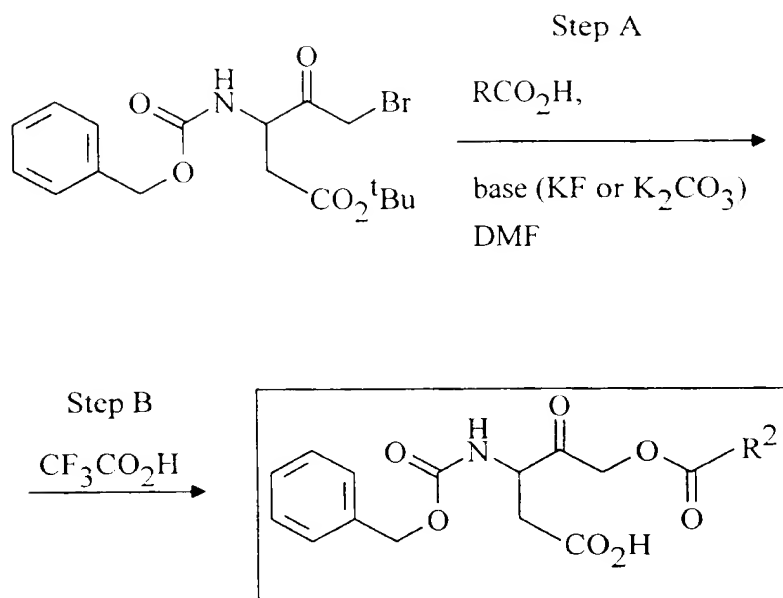
3-[2-(2-Benzyloxycarbonylamino-4-carboxy-butyrylamino)-3-methyl-butyrylamino]-5-(2-benzyl-3-phenyl-propionyloxy)-4-oxo-pentanoic acid;

3-{2-[4-Carboxy-2-(3-phenyl-propionylamino)-butyrylamino]-3-methyl-butyrylamino}-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid; and

20 3-[2-(2-Acetylamino-4-carboxy-butyrylamino)-3-methyl-butyrylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid.

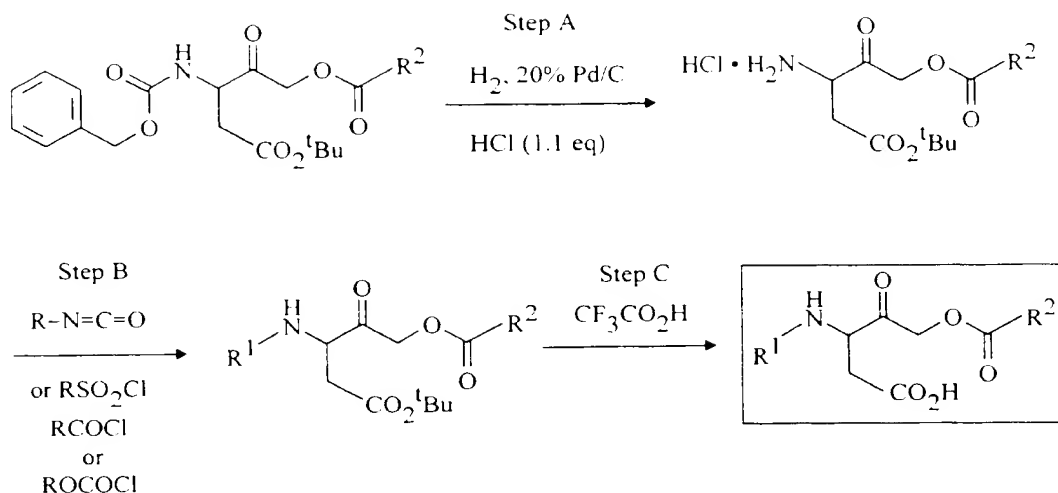
The above-described aspartate ester ICE inhibitors can be made according to the following Schemes 1 through 11:

Scheme 1



- 3-Benzyloxycarbonylamino-5-bromo-4-oxo-pentanoic acid tert-butyl ester, also known as Z-Asp(OtBu)-bromomethyl ketone, can be purchased commercially or prepared according to the procedure of Dolle, et al., *J. Med. Chem.*, 1994;37:563-564. This methylbromo ketone is treated with an appropriately substituted carboxylic acid and a base such as potassium fluoride. Alternately, other bases such as potassium carbonate, cesium carbonate, or potassium t-butoxide could be used. The reagents should be mixed in dimethyl formamide (DMF), dimethylacetamide (DMA), dimethyl sulfoxide (DMSO), acetonitrile or other appropriate solvent and stirred at room temperature for 8 to 24 hours. The t-butyl ester protecting group can be removed in acidic media, preferably trifluoroacetic acid, to produce the carbobenzoxy aspartyl esters shown in Scheme 1.

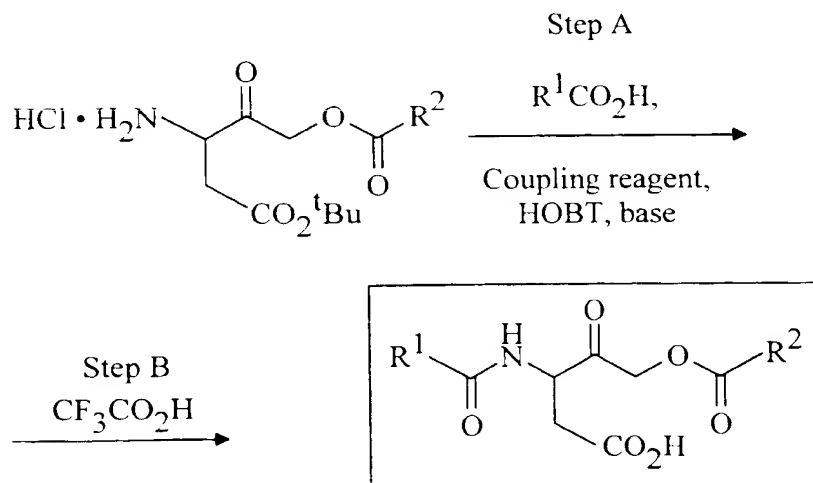
Scheme 2



- 5 A mixture of an appropriately substituted acyloxymethyl ketone of a carbobenzoxy aspartyl t-butyl ester was hydrogenated with an equivalent of hydrochloric or other acid in the presence of a catalyst such as palladium on carbon to yield the amine salt. The salt can be acylated with an appropriately substituted isocyanate, sulfonyl chloride, chloroformate or phenylpropionyl chloride to afford the N-substituted derivatives. These isocyanates, sulfonyl chlorides, or chloro formates can be purchased commercially or synthesized by
- 10 methods described in the chemical literature. The t-butyl ester protecting group can be removed in the final step using acidic media, preferably trifluoroacetic acid, to produce the acyloxy methylketone derivatives shown in Scheme 2.

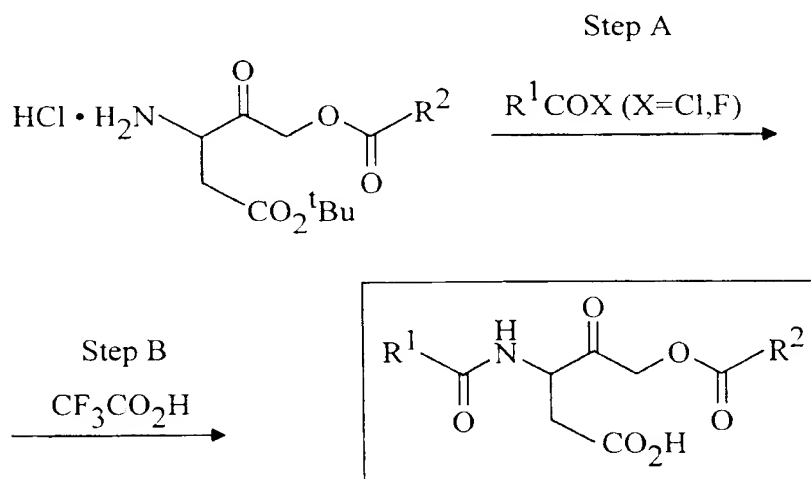
Scheme 3

15



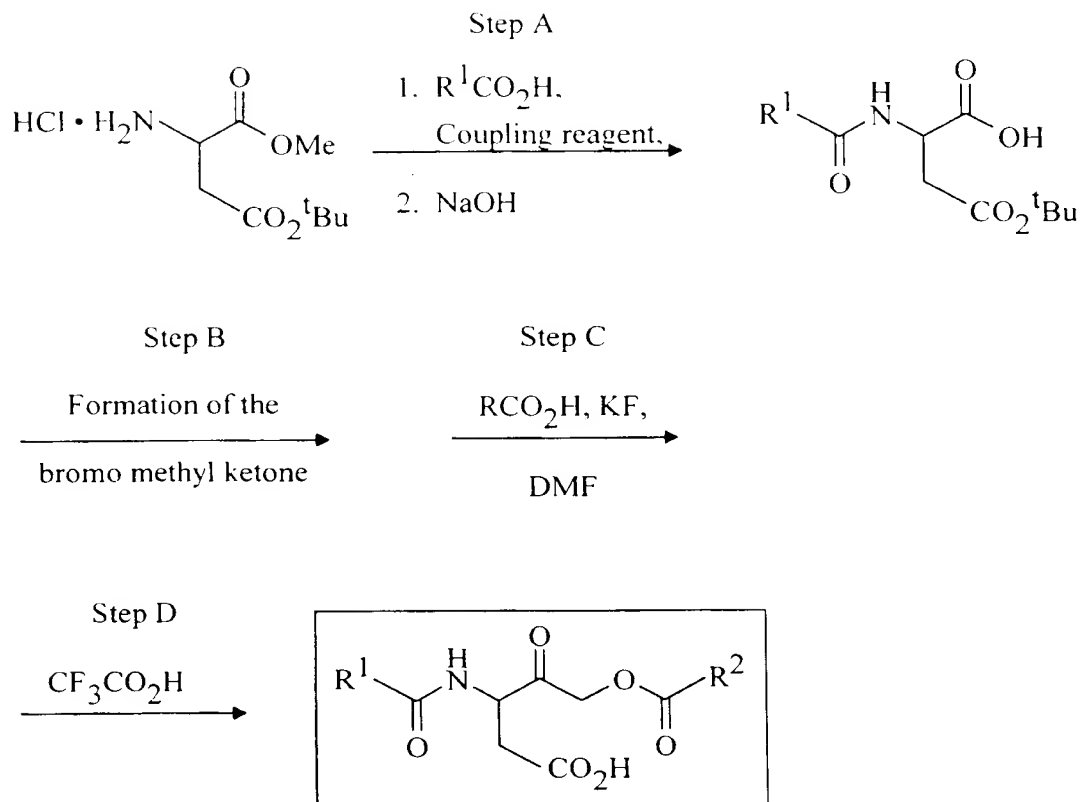
The amine salt of the acyloxymethyl ketone of Z-Asp(Ot-Bu)OH was synthesized and treated with an appropriately substituted carboxylic acid and coupling reagent. The coupling agent may be, but is not limited to, such reagents as 1,3-dicyclohexylcarbodiimide (DCC), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI), 1,1'-carbonyldiimidazole (CDI), 1,1'-carbonylbis(3-methylimidazolium) triflate (CBMIT), isobutylchloroformate, benzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophosphate (BOP), 2-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TDBTU), and 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU). 1-Hydroxybenzotriazole hydrate should be added to the reaction to improve yield and limit isomerization and base, preferably an amine such as trimethyl amine or methyl morpholine should be added as an acid scavenger. The resulting amide product was treated with acidic media, preferably trifluoroacetic acid, to remove the t-butyl ester and produce the final products as described in Scheme 3.

Scheme 4



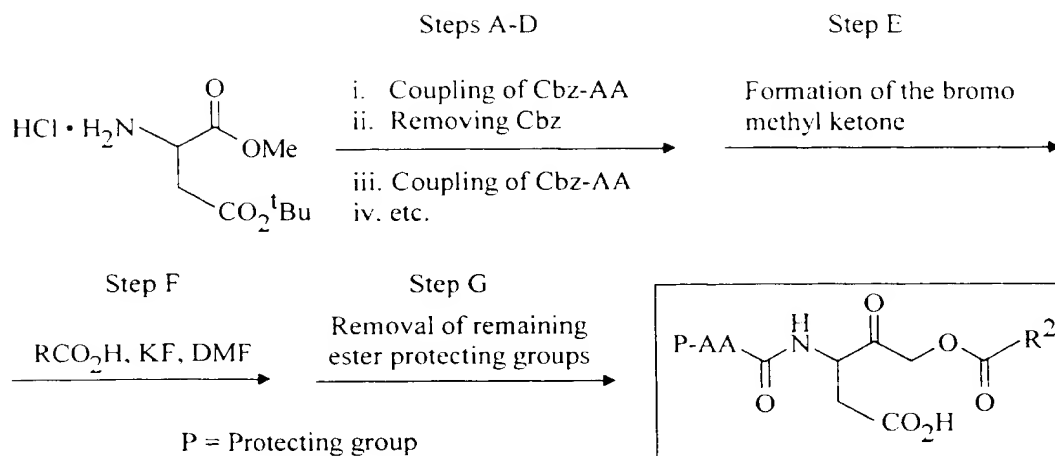
The amine salt of the acyloxymethyl ketone of Cbz-Asp(OtBu)OH was synthesized and treated with an appropriately substituted acid chloride or acid fluoride to generate an amide product. The acid chlorides were purchased commercially or were prepared by treating carboxylic acids with agents such as thionyl chloride, phosphorous tribromide, or oxalyl chloride/DMF. The acid fluorides were prepared by treating a carboxylic acid with cyanuric fluoride. The penultimate amide product was treated with acidic media preferably trifluoroacetic acid to remove the t-butyl ester and afford the final products as described in Scheme 4.

Scheme 5



- The hydrochloride salt of H-Asp(OtBu)OMe was treated with an appropriately substituted carboxylic acid and coupling reagent. 1-Hydroxybenzotriazole hydrate should be added to the reaction to improve yield and limit isomerization and base, preferably an amine such as trimethyl amine or methyl morpholine should be added as an acid scavenger. The resulting amide product was treated with an alkaline reagent such as sodium hydroxide to hydrolyze the methyl ester to the carboxylic acid. The resulting acid was treated with a chloroformate such as isobutylchloroformate, followed by diazomethane and then hydrobromic acid to afford the methyl bromo ketone. Treatment of the methylbromo ketone with an appropriately substituted carboxylic acid and a base such as potassium fluoride produced the desired acyloxymethyl ketones which were deprotected with trifluoroacetic acid to afford the final compounds as described in Scheme 5.

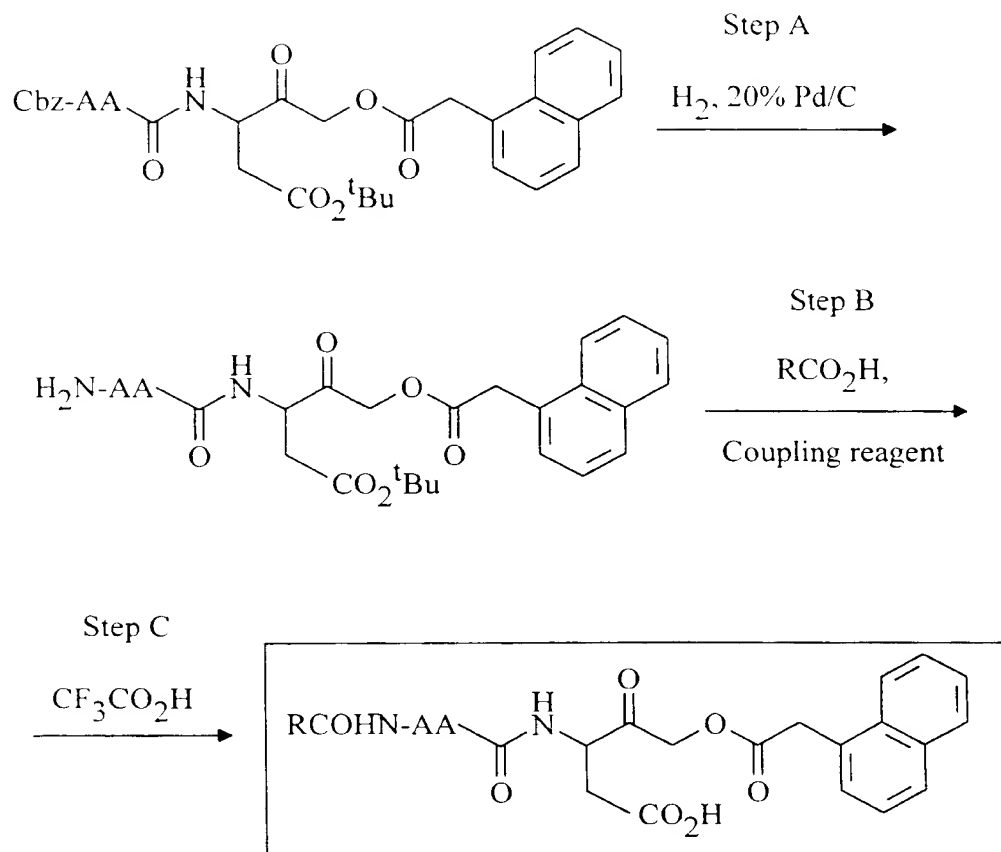
Scheme 6



- The hydrochloride salt of H-Asp(OtBu)OMe was treated with an appropriately protected amino acid and coupling reagent. 1-Hydroxybenzotriazole hydrate should be added to the reaction to improve yield and limit isomerization and base, preferably an amine such as trimethyl amine or methyl morpholine should be added as an acid scavenger. The resulting amide product was treated with an alkaline reagent such as sodium hydroxide to hydrolyze the methyl ester to the carboxylic acid. The Cbz-amine protecting group was removed using standard catalytic hydrogenation conditions and coupling of another protected amino acid can proceed as described above. This process was repeated until the peptide was the desired length. The resulting peptide product was treated with an alkaline reagent such as sodium hydroxide to hydrolyze the methyl ester to the carboxylic acid. The resulting acid was subsequently treated with a chloroformate such as isobutylchloroformate, followed by diazomethane and then hydrobromic acid to afford the methylbromo ketone. Treatment of the methylbromo ketone with an appropriately substituted carboxylic acid and a base such as potassium fluoride produced the desired acyloxymethyl ketones which were deprotected with trifluoroacetic acid to afford the final compounds as described in Scheme 6.

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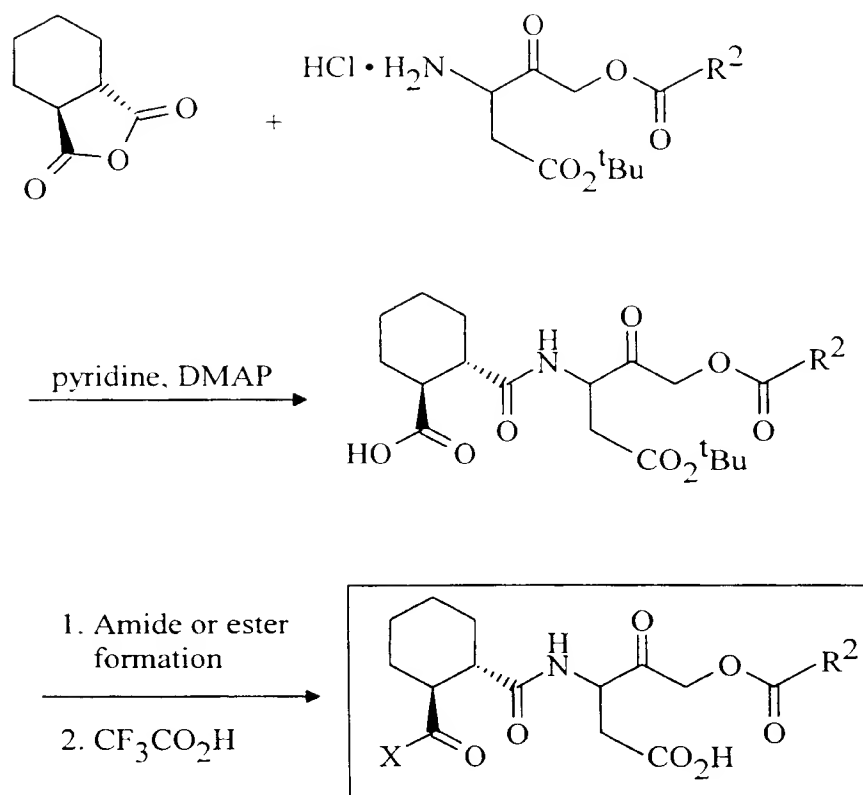
Scheme 7



- The appropriately substituted acyloxymethyl ketone of a protected amino acid was synthesized. The Cbz-amine protecting group was removed using standard catalytic hydrogenation conditions, and the amine product was treated with an appropriately substituted carboxylic acid and a coupling reagent. 1-Hydroxybenzotriazole hydrate should be added to the reaction to improve yield and limit isomerization and base, preferably an amine such as trimethyl amine or methyl morpholine should be added as an acid scavenger. The penultimate amide product was treated with acidic media preferably trifluoroacetic acid to remove the t-butyl ester and afford the final products as described in Scheme 7.

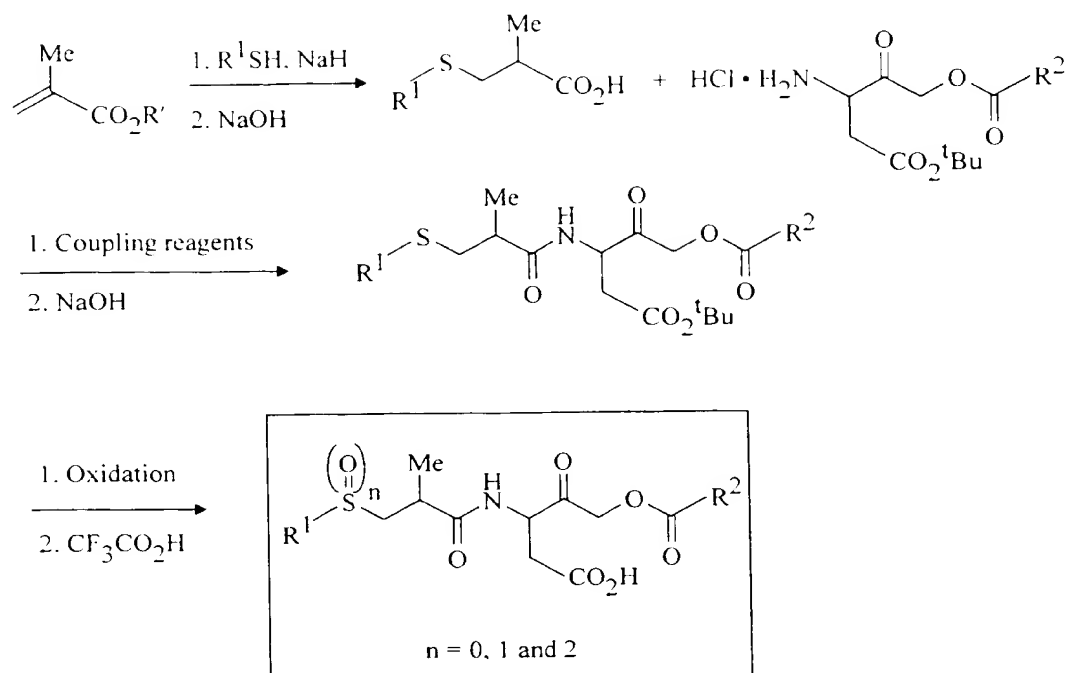
-62-

Scheme 8

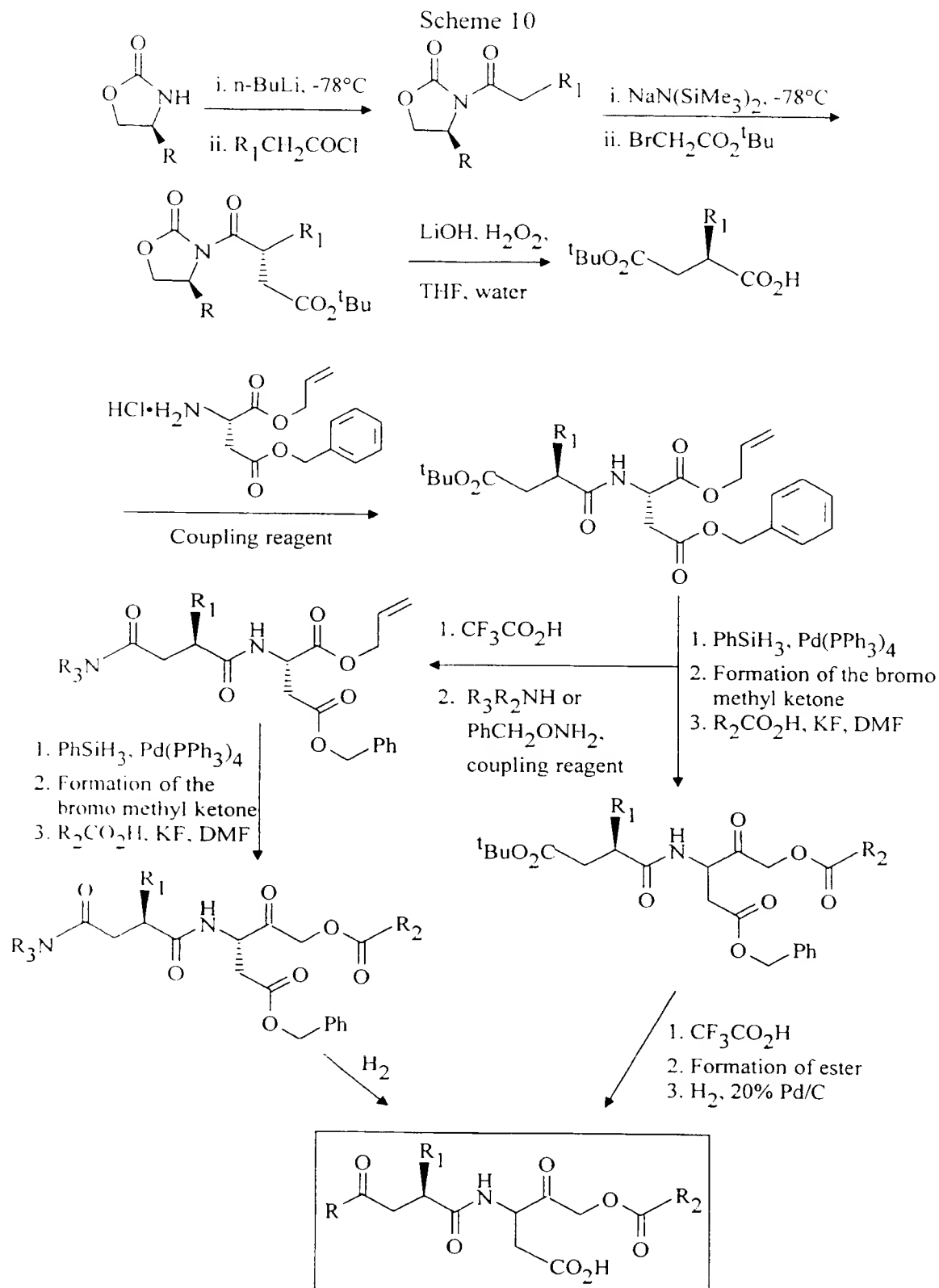


Trans-1,2-cyclohexanedicarboxylic anhydride was treated with the amine salt of an appropriately substituted acyloxymethyl ketone of aspartyl t-butyl ester in the presence of pyridine and 4-dimethylaminopyridine (DMAP) to yield the amide product. The carboxylic acid can be functionalized with appropriately substituted amines or alcohols and standard coupling reagents to afford amide and ester products. The penultimate product was treated with acidic media, preferably trifluoroacetic acid, to remove the t-butyl ester and afford the final products as described in Scheme 8.

Scheme 9



- Methyl methacrylate was treated with the anion of an appropriately substituted sulfide to afford the Michael adduct which was hydrolyzed in basic media such as sodium hydroxide to produce the carboxylic acid. This acid was combined with the amine salt of the acyloxymethyl ketone of aspartyl t-butyl ester and a coupling reagent to obtain the amide product. If the sulfide (where $n = 0$) is the desired product, no oxidation step is employed, and the amide t-butyl ester is deprotected in acidic media, preferably trifluoroacetic acid, to afford the final product. Alternately, if the sulfoxide ($n = 1$) or sulfone ($n = 2$) is the final product, the amide intermediate is treated with an oxidizing agent which may be, but is not limited to, m-chloroperbenzoic acid, potassium monoperoxysulfate, or sodium perborate to obtain the desired oxidized product. The t-butyl ester of the penultimate intermediate was deprotected in acidic media, preferably trifluoroacetic acid, to afford the final compounds as described in Scheme 9.



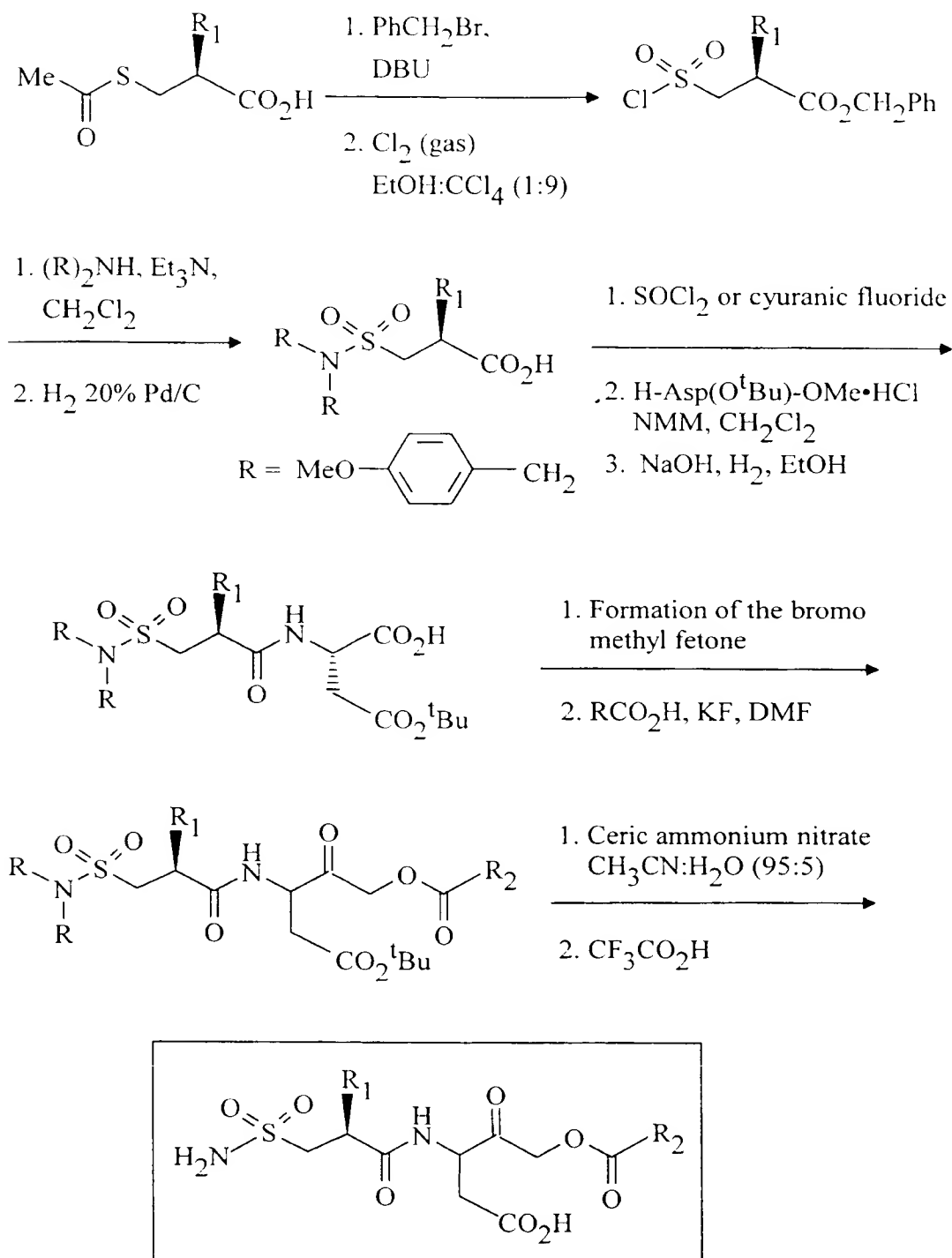
A 4-substituted-2-oxazolidinone chiral auxiliary as described by Evans, et al., *J. Org. Chem.*, 1985;50:1830 was mixed with a base, such as but not limited to, n-butyl

lithium followed by treatment with an appropriately substituted acid chloride or other activated carboxylic acid to afford the N-acylated product. This product was subsequently treated with a base such as, but not limited to, sodium bis(trimethylsilyl)amide and t-butyl bromoacetate to produce the alkylated chiral product. The chiral auxiliary was removed using lithium hydroxide and hydrogen peroxide to obtain the chiral acid. Treatment of the acid with the amine salt of H-Asp(OBz)O-allyl and a coupling reagent afforded the succinyl amide product.

At this stage of the process, the product can be elaborated in one of two ways. First the t-butyl ester was removed in acidic media, preferably trifluoroacetic acid, and the resulting acid was coupled with an appropriately substituted amine in the presence of a coupling reagent to form a new amide product. The allyl ester was removed with phenylsilane and tetrakis(triphenyl-phosphine)palladium or other Pd(0) catalyst to obtain the carboxylic acid, and the acid was converted to the methylbromo ketone and subsequently to the acyloxymethyl ketone. The penultimate intermediate was subjected to catalytic hydrogenation to remove the benzyl ester and afford the final amide products as described in Scheme 10.

Alternatively, in a second route to the final products, the allyl ester is removed using phenylsilane and tetrakis(triphenylphosphine)palladium or other Pd(0) catalyst to obtain the carboxylic acid. This acid is converted to the methylbromo ketone and subsequently to the acyloxymethyl ketone. Removal of the t-butyl ester of the acyloxymethyl ketone with trifluoroacetic acid and subsequent conversion of the resulting carboxylic acid to the ester resulted in a new ester product. The esterification can be accomplished using a variety of literature techniques which includes but is not limited to treatment of the carboxylic acid with an appropriately substituted alcohol in the presence of a coupling reagent. The penultimate intermediate was subjected to catalytic hydrogenation to remove the benzyl ester and afford the final ester products as described in Scheme 10.

Scheme 11



The appropriately substituted S-acetyl mercapto carboxylic acid was treated with benzyl bromide and 1,8-diazobicyclo[5.4.0]undec-7-ene (DBU) to produce the benzyl ester
 5 which was subsequently reacted with chlorine gas to yield the sulfonyl chloride. The

sulfonyl chloride was treated with N,N-bis(p-methoxybenzyl)amine to afford the sulfonamide which was subjected to catalytic hydrogenation to obtain the intermediate carboxylic acid. The acid was activated using cyranic fluoride which was then mixed with the amine salt of H-Asp(Ot-Bu)OMe to produce the amide product. The methyl ester was hydrolyzed with sodium hydroxide, and the carboxylic acid was elaborated to the acyloxymethyl ketone. The p-methoxybenzyl protecting groups of the sulfonamide were removed using oxidizing conditions preferably, but not limited to ceric ammonium nitrate, and the t-butyl ester protecting group was removed in acidic media preferably with trifluoroacetic acid to afford the desired sulfonamide products as described in Scheme 11.

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As used herein, the term "alkyl" means a straight or branched chain hydrocarbon. Representative examples of alkyl groups are methyl, ethyl, propyl, isopropyl, isobutyl, butyl, tert-butyl, sec-butyl, pentyl, and hexyl.

The term "alkoxy" means an alkyl group attached to an oxygen atom.

15 Representative examples of alkoxy groups include methoxy, ethoxy, tert-butoxy, propoxy, and isobutoxy.

The term "halogen" includes chlorine, fluorine, bromine, and iodine.

The term "aryl" means an aromatic hydrocarbon. Representative examples of aryl groups include phenyl and naphthyl.

20 The term "heteroatom" includes oxygen, nitrogen, sulfur, and phosphorus.

The term "heteroaryl" means an aryl group wherein one or more carbon atom of the aromatic hydrocarbon has been replaced with a heteroatom. Examples of heteroaryl groups include furan, thiophene, pyrrole, thiazole, pyridine, pyrimidine, pyrazine, benzofuran, indole, coumarin, quinoline, isoquinoline, and naphthyridine.

25 The term "cycloalkyl" means a cyclic alkyl group. Examples of cycloalkyl groups include cyclopropane, cyclobutane, cyclopentane, and cyclohexane.

The term "heterocycle" means a cycloalkyl group on which one or more carbon atom has been replaced with a heteroatom. Examples of heterocycles include piperazine, morpholine, and piperidine.

30 The aryl, heteroaryl, or cycloalkyl groups may be substituted with one or more substituents, which can be the same or different. Examples of suitable substituents include alkyl, alkoxy, thioalkoxy, hydroxy, halogen, trifluoromethyl, amino, alkylamino, dialkylamino, -NO₂, -CN, -CO₂H, -CO₂alkyl, -SO₃H, -CHO, -COalkyl, -CONH₂, -CONH-alkyl, -CONHR^q, -CON(alkyl)₂, -(CH₂)_n-NH₂, -OH, -CF₃, -OC₁-C₆alkyl, -
35 (CH₂)_n-NH-alkyl, -NHR^q, -NHCOR^q, phenyl, -(CH₂)_nOH, -(CH₂)_nC(O)NH₂, or -(CH₂)_nCO₂H, where n is 1 to 5 and R^q is hydrogen or alkyl.

The symbol "-" means a bond.

Examples of other caspase family inhibitors contemplated for use in the invention include Ich-1 inhibitors such as those described in PCT Publication No. 97/27220.

The term "phosphodiesterase IV inhibitor" is intended to refer to agents that inhibit the activity of the enzyme phosphodiesterase IV. Examples of phosphodiesterase IV inhibitors are known in the art and include 4-arylpyrrolidinones, such as rolipram (see *e.g.*, Sekut, L. *et al.* (1995) *Clin. Exp. Immunol.* 100:126-132), nitraquazone (see *e.g.*, Van Wauwe, J. *et al.* (1995) *Inflamm. Res.* 44:400-405), denbufylline, tibenelast (see *e.g.*, Banner, K.H. *et al.* (1996) *Br. J. Pharmacol.* 119:1255-1261), CP-80633 (see *e.g.*, Cohan, V.L. *et al.* (1996) *J. Pharmacol. Exp. Therap.* 278:1356-1361) and quinazolininediones, such as CP-77059 (see *e.g.*, Sekut, L. *et al.* (1995) *Clin. Exp. Immunol.* 100:126-132).

The term "beta-2 agonist" is intended to refer to agents that stimulate the beta-2 adrenergic receptor. Examples of beta-2 agonists are known in the art and include salmeterol (see *e.g.*, Sekut, L. *et al.* (1995) *Clin. Exp. Immunol.* 99:461-466), fenoterol and isoproterenol (see *e.g.*, Severn, A. *et al.* (1992) *J. Immunol.* 148:3441-3445).

The term "STAT4" is intended to refer to a transcription factor involved in IL-12 responses (see *e.g.*, Thierfelder, W.E. *et al.* (1996) *Nature* 382:171-174; Kaplan, M.H. *et al.* (1996) *Nature* 382:174-177). The term "STAT4 inhibitor" refers to an agent that inhibits the activity of the STAT4 transcription factor such that responses to IL-12 are inhibited.

The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active determinants of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen. The term "antibody" is further intended to include bispecific and chimeric molecules having at least one antigen binding determinant derived from an antibody molecule. Furthermore, although the H and L chains of an Fv fragment are encoded by separate genes, a synthetic linker can be made that enables them to be made as a single protein chain (known as single chain antibody, sAb; Bird *et al.* 1988 *Science* 242:423-426; and Huston *et al.* 1988 *PNAS* 85:5879-5883) by recombinant methods. Such single chain antibodies are also encompassed within the term "antibody", and may be utilized as binding determinants in the design and engineering of a multispecific binding molecule.

The term "antibody fragment" as used herein refers to an active fragment of an antibody that retains the ability to bind (immunoreact with) an antigen. Examples of antibody fragments include: a Fab fragment consisting of the V_L, V_H, C_L and C_{H1} domains; an Fd fragment consisting of the V_H and C_{H1} domains; an Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody; a dAb fragment (Ward *et al.*, 1989 *Nature* 341:544-546) consisting of a V_H domain; an isolated complementarity determining region (CDR); and an F(ab')₂ fragment, a bivalent fragment comprising two Fab' fragments linked by a disulfide bridge at the hinge region. These antibody fragments are obtained using conventional techniques well-known to those with

skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

The term "engineered binding protein" as used herein is intended to include molecules derived from an antibody or other binding molecule (*e.g.*, a receptor or ligand) that retain a desired binding specificity but that have been engineered by recombinant DNA techniques and/or are expressed using recombinant DNA techniques. Examples of engineered binding proteins include soluble and truncated forms of receptors, dimers of receptors (*e.g.*, p40 IL-12 receptor dimers), and modified or mutated forms of antibodies, ligands or receptors selected using combinatorial libraries (*e.g.*, phage display library techniques).

The term "NK cell antagonist" as used herein is intended to include antibodies, antibody fragments and engineered binding proteins that are capable of depleting NK/NK-like cells when administered to a subject. Examples of NK cell antagonists include anti-asialo-GM1 antibodies and NK1.1 antibodies.

The terms "steroid resistant disease" and "steroid resistant subject" as used herein are intended to refer to diseases and subjects that do not respond significantly to corticosteroid therapy prior to treatment in accordance with the methods of the invention. Steroid resistance is also referred to as steroid refractoriness.

The term "immunoinflammatory disease or disorder" is intended to include inflammatory diseases and disorders in which immune cells and/or cytokines are involved in the pathophysiology of the disease or disorder. The term "acute inflammatory disorder" is intended to include disorders, and episodes of disorders, characterized by rapid onset of symptoms associated with an inflammatory response and relatively short duration of symptoms, whereas a "chronic inflammatory disorder" is intended to include disorders characterized by the continued presence of symptoms associated with an inflammatory response and ongoing duration of symptoms.

I. Methods of the Invention

In one embodiment, the invention provides a method for modulating responsiveness to a corticosteroid in a subject, comprising administering to the subject:

an agent which antagonizes a target that regulates production of interferon- γ (IFN- γ) in the subject, the agent being administered at a dosage and by a route sufficient to inhibit production of IFN- γ in the subject; and

a corticosteroid,

such that responsiveness of the subject to the corticosteroid is modulated as compared to when a corticosteroid alone is administered to the subject.

In one embodiment, the method involves administration of an agent that is an IL-18 antagonist. The IL-18 antagonist is administered to the subject at a dosage and by a route sufficient to inhibit IL-18 activity in the subject. The IL-18 antagonist can act, for example, by inhibiting IL-18 synthesis in the subject, by inhibiting IL-18 cytokine activity in the subject, by inhibiting interaction of IL-18 with an IL-18 receptor or by inhibiting the activity of an IL-18 receptor.

In a preferred embodiment, the IL-18 antagonist is an inhibitor of a caspase family protease. Caspase family proteases, and in particular ICE, process the precursor form of IL-18 to the mature (*i.e.*, active) form (see *e.g.*, Example 4). Accordingly, although not intending to be limited by mechanism, a caspase family protease inhibitor is thought to antagonize IL-18 activity by inhibiting the processing of IL-18 from its precursor form to its mature (*i.e.*, active) form.. A preferred caspase family protease inhibitor for use in the methods of the invention is an ICE inhibitor. Additionally or alternatively, other caspase family proteases that are capable of cleaving precursor IL-18 to mature IL-18 (such as Ich-2 (caspase-4) and ICE_{rel}III (caspase-5)), can be inhibited. Chemical agents that can inhibit the activity of ICE and other caspase family proteases are known in the art, including peptidyl derivatives, azaaspartic acid analogs and gamma-pyrone-3-acetic acid (see *e.g.*, U.S. Patent No. 5,411,985, U.S. Patent No. 5,430,128, U.S. Patent No. 5,434,248, U.S. Patent No. 5,565,430, U.S. Patent No. 5,416,013, PCT Publication WO 94/00154, PCT Publication WO 93/16710, PCT Publication WO 93/14777, PCT Publication WO 93/05071, PCT Publication WO 95/35308, European Patent Application EP 547 699 and European Patent Application EP 519 748). Additional suitable inhibitors of ICE and other caspase family inhibitors are disclosed in U.S. Application Serial No. 08/700,716 and U.S. Provisional Applications Serial Nos. 60/028,322, 60/028,324, 60/028,313 and 60/028,323. The exact dosage and regimen for administering an inhibitor of ICE or an ICE-family protease will necessarily depend upon the needs of the subject being treated, the type of treatment, the efficacy of the compound and the degree of disease severity in the subject. However, a nonlimiting example of a dosage range for an inhibitor of ICE and other caspase family proteases is from about 0.05 to about 150 mg/kg body weight/day.

In other embodiments, the IL-18 antagonist is an antibody, antibody fragment, or engineered binding protein that binds IL-18 or an IL-18 receptor. Such binding agents can be prepared by standard methods known in the art for making poly- and monoclonal antibodies and recombinant binding proteins and are described further in, for example, European Patent Application 692 536, European Patent Application 712 931, PCT Publication WO 97/24441 and PCT Publication WO 97/44468.

In another embodiment, the method of the invention involves administration of an agent that is an IL-12 antagonist. The IL-12 antagonist is administered to the subject at a

dosage and by a route sufficient to inhibit IL-12 activity in the subject. The IL-12 antagonist can act, for example, by inhibiting IL-12 synthesis in the subject, by inhibiting IL-12 cytokine activity in the subject, by inhibiting interaction of IL-12 with an IL-12 receptor or by inhibiting the activity of an IL-12 receptor.

5 In one embodiment, the IL-12 antagonist is an antibody, antibody fragment, or engineered binding protein that binds IL-12 or IL-12 receptor. A preferred IL-12 antagonist is an anti-IL-12 monoclonal antibody. Such antibodies have been described in the art (see *e.g.*, Chizzonite, R. *et al.* (1991) *J. Immunol.* 147:1548-1556). The ability of anti-IL-12 monoclonal antibodies to inhibit disease responses also has been described in the
10 art (see *e.g.*, Leonard, J.P. *et al.* (1995) *J. Exp. Med.* 181:381-386; Neurath, M.F. *et al.* (1995) *J. Exp. Med.* 182:1281-1290). Another type of IL-12 antagonist is a p40 homodimer (see *e.g.*, Gillesen, S. *et al.* (1995) *Eur. J. Immunol.* 25:200-206; Gately, M.K. *et al.* (1996) *Ann. NY Acad. Sci.* 795:1-12; Ling, P. *et al.* (1995) *J. Immunol.* 154:116-127). Yet another type of IL-12 antagonist is a low affinity form of an IL-12 receptor, as described in
15 European Patent Application EP 638 644 and U.S. Patent No. 5,536,657.

Nonlimiting examples of IL-12 antagonists for use in the methods of the invention include mono- and polyclonal antibodies and fragments thereof, chimeric antibodies and fragments thereof, soluble IL-12 receptors and fragments thereof, reactive peptides or fragments thereof, chemically or genetically modified peptides of IL-12, subunits of IL-12
20 and fragments thereof, homopolymers of IL-12 subunits and fragments thereof, and small organic molecules designed to inhibit the bioactivity of IL-12 or IL-12 receptors. The preparation of IL-12 antagonists, including: (i) species that bind IL-12 or biologically active fragments thereof, and (ii) species that interfere with the binding of IL-12 to receptors or other binding proteins, have been described in the art (see *e.g.*, PCT
25 Publication WO 95/24918 by Leonard *et al.*, the contents of which are expressly incorporated herein by reference; see also Presky, D.H. *et al.* (1995) *Res. Immunol.* 146:439-445).

In another embodiment, an IL-12 antagonist used in the method of the invention is an agent that stimulates cyclic AMP (cAMP) production in cells that produce IL-12.
30 Production of IL-12 has been shown to be inhibited by increased intracellular production of cAMP (see *e.g.*, van der Pouw Kraan *et al.* (1995) *J. Exp. Med.* 181:775-779). Examples of agents that can be used to stimulate intracellular cAMP production include phosphodiesterase IV inhibitors and beta-2 agonist. As demonstrated in Example 3, administration of a phosphodiesterase IV inhibitor in a septic shock model inhibits LPS-
35 induced IL-12 production. Examples of suitable phosphodiesterase IV inhibitors for use in the methods of the invention include rolipram, denbufylline, tibenelast, nitraquazone and CP-80633. Examples of beta-2 agonists for use in the methods of the invention include salmeterol, fenoterol and isoproterenol. The exact dosage and regimen for administering a

phosphodiesterase IV inhibitor or a beta-2 agonist will necessarily depend upon the needs of the subject being treated, the type of treatment, the efficacy of the compound and the degree of disease severity in the subject. However, a nonlimiting example of a dosage range for phosphodiesterase IV inhibitors or beta-2 agonists is from about 0.05 to about 150 mg/kg body weight/day. In a preferred embodiment, an agent that stimulates cyclic AMP (cAMP) production (*e.g.*, a phosphodiesterase IV inhibitor or a beta-2 agonist) is administered systemically (*e.g.*, orally or intravenously) to inhibit production of IL-12 systemically by monocytes and macrophages.

In another embodiment, an IL-12 antagonist used in the method of the invention is a STAT4 inhibitor. STAT4 is a transcription factor that has been shown to be involved in IL-12 responses (see *e.g.*, Thierfelder, W.E. *et al.* (1996) *Nature* 382:171-174; Kaplan, M.H. *et al.* (1996) *Nature* 382:174-177). Accordingly, IL-12 responses in a subject can be inhibited through administration of a STAT4 inhibitor.

Other inhibitors of IL-12 activity that are known in the art also can be used in the methods of the invention. For example, PCT Publication WO 96/40093 discloses biphenyl derivatives for antagonizing IL-12 induced immune responses. Such biphenyl derivatives can be used as IL-12 antagonists in the methods of the invention.

In another embodiment, the method of the invention involves administration of an agent that is an NK cell antagonist. The NK cell antagonist is administered to the subject at a dosage and by a route sufficient to inhibit IFN- γ activity in the subject. Preferably, the NK cell antagonist is an antibody, antibody fragment, or engineered binding protein that specifically binds to NK/NK-like cells such that the cells are depleted or eliminated in a subject. Accordingly, preferred NK cell antagonists bind to specific surface markers present on NK/NK-like cells. Particular preferred NK cell antagonists are anti-asialo-GM1 antibodies and NK1.1. antibodies, which have been shown to be effective in depleting NK/NK-like activity from a subject (see Example 10; Axelsson, L-G. *et al.* (1996) *Inflamm. Res.* 45:181-191; Heremans, H. *et al.* (1994) *Eur. J. Immunol.* 24:1155-1160).

Other antibodies that target surface markers that identify NK/NK-like cells include antibodies reactive with Fc-IgG receptors B73.1 and Leu 11 (CD16) (Lancer, L.L. *et al.* (1983) *J. Immunol.* 131:1789-1796; Perussia, B. *et al.* (1983) *J. Immunol.* 130:2133-2141), Leu 7 (anti-HNK1, which identify 40-60% of NK cells; Abo, T. and Balch, C.M. (1981) *J. Immunol.* 127:1024-1029), and OKT11 (CD2, which identify 50% or more of NK cells; Lancer, L.L. *et al. supra*; Perussia, B. *et al., supra*). Other NK cell-specific surface antigens, and antibodies thereto, that have been described include the DX1 antigen (see PCT Publication WO 95/02611), the PEN5-alpha and PEN5-beta glycoprotein pair (see PCT Publication WO 95/06247) and the NKB1 antigen (see PCT Publication WO 95/20604).

The exact dosage and regimen for administering an NK cell antagonist will necessarily depend upon the needs of the subject being treated, the type of treatment, the efficacy of the compound and the degree of disease severity in the subject. However, a nonlimiting example of a dosage range for anti-NK/NK-like cell antibodies is from about 0.01 to about 150 mg/kg body weight/day. A single dosage of antibody may be sufficient to deplete or eliminate NK/NK-like cell activity or, alternatively, multiple dosages may be given as needed to deplete or eliminate NK/NK-like cell activity. Preferably, the NK antagonist is administered by an intravenous or intraperitoneal route.

In the methods of the invention, an agent which antagonizes a target that regulates production of interferon- γ (IFN- γ) is administered to a subject in combination with one or more corticosteroids. The term "in combination with" a corticosteroid is intended to include simultaneous administration of the agent and the corticosteroid, administration of the agent first, followed by the corticosteroid and administration of the corticosteroid first, followed by the agent. Any of the therapeutically useful corticosteroids known in the art can be used in the methods of the invention. Corticosteroids are typically classified by the duration of their tissue effects: short acting compounds (*e.g.*, beclomethasone, flunisolide, hydrocortisone, cortisone), intermediate acting compounds (*e.g.*, prednisone, prednisolone, methylprednisolone, triamcinolone, deflazacort) and long-acting compounds (*e.g.*, dexamethasone, beta methasone). One or more corticosteroids can be administered to the subject by a route and at a dosage effective to achieve the desired therapeutic results. Examples of suitable routes of delivery include intravenous administration, oral administration, topical administration, administration by inhalation (*e.g.*, bronchial administration), and local injection (*e.g.*, intra-joint). The exact dosage and regimen for administering a corticosteroid to the subject will necessarily depend upon the needs of the subject being treated, the type of treatment, the efficacy of the compound and the degree of disease severity in the subject. However, a nonlimiting example of a dosage range for corticosteroids is from about 0.05 mg/day to about 1 gm/day, depending upon the particular corticosteroid used. Certain preferred dosage regimens utilize alternate day administration (*e.g.*, high dose intravenous pulse therapy).

Corticosteroid formulations suitable for administration are well known in the art and commercially available. For example, dexamethasone acetate, 16 mg/ml aqueous suspension, is suitable for intramuscular injection in the treatment of rheumatoid, dermatological, ophthalmic, gastrointestinal, hematologic, neoplastic, allergic conditions and collagen disorders. Nonlimiting examples of dosages include 0.8 mg, 1.6 mg, 4 mg and 16 mg of dexamethasone per injection.

Hydroxycortisone is available as a sterile aqueous solution for intravenous, intramuscular, and subcutaneous injection and is a potent anti-inflammatory agent for conditions such as osteoarthritis, rheumatoid arthritis, juvenile rheumatoid arthritis, acute

and chronic bursitis. The preferred initial dosages can be from 15 mg to 250 mg per human subject per day. Preferred dosages are oral or parenteral, and can be administered in half the daily dosage, administered twice per day, or other multiples. Hydrocortisone injection can be added to sodium chloride injection or dextrose injection and administered by

5 intravenous drip. Hydrocortisone valerate, 0.2% by weight, is formulated as a cream for topical use under the name Westcort. Preferred dosages comprise application to affected areas several times daily as thin films.

Beconase (beclomethasone) is available for inflammation of the nasal passages and sinuses, for example, as 8.4 mg for 200 metered spray doses in a 0.042% aqueous

10 suspension, delivered in metered doses of 100 mg containing 42 µg per metered dose, such that daily nasal delivery consists of preferably 42 µg per nostril, 84 µg per nostril, 168 µg per nostril, 336 µg per nostril, 672 µg per nostril, or 1,344 µg per nostril. It is preferably delivered, for example, in an aqueous medium in suspension with microcrystalline cellulose, carboxymethylcellulose sodium, dextrose, benzalkonium chloride, polysorbate

15 80, and 0.25% v/w phenylethyl alcohol. Additional propellants and media are included in some formulations.

In certain embodiments in which an agent of the invention is coadministered with a corticosteroid, the agent is administered systemically to regulate IFN-γ production systemically while the corticosteroid is administered either locally or systemically. For

20 example, in certain embodiments when a phosphodiesterase IV inhibitor or a beta-2 agonist is administered together with a corticosteroid, the phosphodiesterase IV inhibitor or beta-2 agonist is administered systemically, such as intravenously or orally, and the corticosteroid is administered either systemically or locally. Additionally, in certain embodiments of the methods of the invention, use of a phosphodiesterase IV inhibitor or a beta-2 agonist in

25 combination with a corticosteroid for the treatment of asthma is specifically excluded from the scope of the invention.

The methods of the invention can be used in the treatment of a variety of inflammatory and immunological disorders. For example, in a preferred embodiment, the

30 subject to be treated is suffering from septic shock (*i.e.*, the methods of the invention allow for corticosteroids to be used in the treatment of septic shock). In another preferred embodiment, the subject to be treated is suffering from Crohn's disease. In yet another preferred embodiment, the subject to be treated is suffering from asthma. In still another preferred embodiment, the subject to be treated is suffering from graft-versus-host disease

35 or transplant rejection. In still another preferred embodiment, the subject to be treated is suffering from an autoimmune disease.

In another embodiment, the subject to be treated is suffering from an immunoinflammatory disease or disorder. Non-limiting examples of immunoinflammatory

diseases and disorders that may be treated according to the invention include asthma, adult respiratory distress syndrome, systemic lupus erythematosus, inflammatory bowel disease (including Crohn's disease and ulcerative colitis), multiple sclerosis, insulin-dependent diabetes mellitus, autoimmune arthritis (including rheumatoid arthritis, juvenile rheumatoid arthritis, psoriatic arthritis), inflammatory pulmonary syndrome, pemphigus vulgaris, idiopathic thrombocytopenic purpura, autoimmune meningitis, myasthenia gravis, autoimmune thyroiditis, dermatitis (including atopic dermatitis and eczematous dermatitis), psoriasis, Sjögren's Syndrome (including keratoconjunctivitis sicca secondary to Sjögren's Syndrome), alopecia areata, allergic responses due to arthropod bite reactions, aphthous ulcer, iritis, conjunctivitis, keratoconjunctivitis, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, drug eruptions (such as Stevens-Johnson syndrome), leprosy reversal reactions, erythema nodosum leprosum, autoimmune uveitis, allergic encephalomyelitis, aplastic anemia, pure red cell anemia, idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active hepatitis, Graves ophthalmopathy, primary biliary cirrhosis, uveitis posterior and interstitial lung fibrosis.

In another embodiment, the subject to be treated is suffering from an acute inflammatory disorder. Examples of acute inflammatory disorders including graft versus host disease, transplant rejection, septic shock, endotoxemia, Lyme arthritis, infectious meningitis (*e.g.*, viral, bacterial, Lyme disease-associated), an acute episode of asthma and acute episodes of an autoimmune disease.

In yet another embodiment, the subject to be treated is suffering from a chronic inflammatory disorder. Nonlimiting examples of chronic inflammatory disorder which can be treated include asthma, rubella arthritis, and chronic autoimmune diseases, such as systemic lupus erythematosus, psoriasis, inflammatory bowel disease, including Crohn's disease and ulcerative colitis, multiple sclerosis and rheumatoid arthritis.

In certain cases, agents that antagonize a particular target that regulates IFN- γ in the subject may be preferred for the treatment of a particular disorder. For example, although not intending to be limited by mechanism, disorders in which IFN- γ is preferentially or predominantly produced by NK cells preferably are treated using an agent that antagonizes IL-18 (such as an ICE inhibitor) or directly antagonizes the NK cells (*i.e.*, an NK cell antagonist, such as an anti-NK/NK-like cell antibody), in combination with a corticosteroid. Alternatively, disorders in which IFN- γ is preferentially or predominantly produced by T cells preferably are treated using an agent that antagonizes IL-12 (*e.g.*, an anti-IL-12 antibody or an agent that stimulates intracellular production of cAMP), in combination with a corticosteroid. In other circumstances, it may be beneficial to use both an IL-18 antagonist and an IL-12 antagonist (*e.g.*, in the treatment of disorders in which IFN- γ production is contributed by both T cells and NK cells).

The agent and the corticosteroid are administered to the subject in need of treatment according to standard routes of drug delivery well known in the art, the particular route and dosage of the agent and the corticosteroid being selected depending upon the needs of the subject being treated, the type of treatment, the efficacy of the compound and the degree of disease severity in the subject. The agent and the corticosteroid are administered at an "effective therapeutic dose", which means that amount of the therapeutic composition which, when administered to a subject produces an amelioration of a disorder in comparison to those subjects which have not been administered the drug. One of ordinary skill in the art can determine and prescribe the effective amount of the therapeutic agents and corticosteroid required. The agents and corticosteroids of the invention are administered to subjects in biologically compatible forms suitable for pharmaceutical administration *in vivo* to produce a desired therapeutic response. By "biologically compatible form suitable for administration *in vivo*" is meant a form of the drug to be administered in which any toxic effects and side effects are outweighed by the therapeutic effects of the composition. Moreover, an agent of the invention that antagonizes a target that regulates production of IFN- γ in a subject is administered to the subject at a dosage and by a route sufficient to inhibit IFN- γ production in the subject. Similarly, an IL-12 antagonist or IL-18 antagonist of the invention is administered to a subject at a dosage and by a route sufficient to inhibit IL-12 activity or IL-18 activity, respectively, in the subject.

Animal models of inflammatory and immunological disorders that are accepted in the art as being models of human disease can be used to evaluate various therapeutic regimens of the invention. For example, the *P.acnes*/LPS model of septic shock described in the Examples can be used to evaluate the efficacy of therapeutic regimens for the treatment of septic shock. Numerous animal models of autoimmune disease are known in the art and can be applied to the methods herein to evaluate the efficacy of therapeutic regimens, nonlimiting examples of which include experimental colitis (see *e.g.*, Neurath, M.F. *et al.* (1995) *J. Exp. Med.* 182:1281-1290), experimental allergic encephalomyelitis (see *e.g.*, Leonard, J.P. *et al.* (1995) *J. Exp. Med.* 181:381-386), collagen-induced arthritis (Banerjee, S. *et al.* (1989) *J. Immunol.* 142:2237-2243) and the human TNF α transgenic model of polyarthritis (see *e.g.*, Keffer, J. *et al.* *EMBO J* (1991) 10:4025-4031). For therapeutic regimens involving inhibition of ICE activity, ICE deficient mice can be used as a model of complete inhibition of ICE activity. Such ICE $-/-$ mice have been described in the art (see *e.g.*, Li, P., *et al.* (1995) *Cell* 80:401-411 and PCT Publication No. WO 96/12025).

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The methods of the invention are useful for modulating corticosteroid responsiveness in a variety of clinical settings. For example, in one embodiment, the methods of the invention are used to reverse steroid resistance in a subject, as compared to

when a corticosteroid alone is administered to the subject. In another embodiment, the methods of the invention are used to increase steroid sensitivity in a subject, as compared to when a corticosteroid alone is administered to the subject. In yet another embodiment, the corticosteroid is administered to a subject according to a schedule that reduces the dosage of the corticosteroid over time and the method ameliorates a steroid rebound effect associated with administration of reduced dosages of the corticosteroid. The ability of the methods of the invention to increase steroid sensitivity (*i.e.*, to have a "steroid sparing effect") may therefore allow for the use of corticosteroid therapy in clinical situations in which such therapy previously has been contraindicated. For example, use of the methods of the invention may allow for corticosteroid therapy in patients that previously could not be treated because of detrimental side effects of corticosteroid therapy, such as young children (*e.g.*, in juvenile rheumatoid arthritis), patients with uncontrolled diabetes and patients with hypertension.

Another aspect of the invention pertains to a method for modulating responsiveness to a corticosteroid in a subject, comprising:
selecting a subject in need of modulation of responsiveness to a corticosteroid; and
administering to the subject an agent which antagonizes a target that regulates production of interferon- γ (IFN- γ) in the subject, the agent being administered at a dosage and by a route sufficient to inhibit production of IFN- γ in the subject,
such that responsiveness of the subject to a corticosteroid is modulated as compared to when a corticosteroid alone is administered to the subject.

The subject that is selected for treatment according to the method can be, for example a subject that is resistant to a corticosteroid prior to administration of the agent. Alternatively, the subject that is selected for treatment can be a subject that is responsive to a corticosteroid prior to administration of the agent but that exhibits increased sensitivity to the corticosteroid after administration of the agent. One examples of such a subject is a patients suffering from a steroid dependent disorder, which disorder can be treated with lower doses of corticosteroids when treated in accordance with the methods of the invention. Another example of such a subject is a patient for whom steroid therapy has been contraindicated due to side effects when the corticosteroid is administered alone but who can tolerate a lower dosage of corticosteroid when the corticosteroid is administered in accordance with the methods of the invention. Still further, the subject that is selected for treatment according to the method can be a subject undergoing corticosteroid therapy but in whom corticosteroid therapy is to be stopped, such that administration of the agent ameliorates a steroid rebound effect in the subject. Agents for antagonizing a target that regulates production of IFN- γ in the subject are as described hereinbefore.

II. Pharmaceutical Compositions

Another aspect of the invention pertains to pharmaceutical compositions for
5 modulating responsiveness to corticosteroids. In one embodiment, the pharmaceutical
composition of the invention comprises an agent which antagonizes a target that regulates
production of interferon- γ (IFN- γ) in the subject, a corticosteroid and a pharmaceutically
acceptable carrier. As discussed above, the target that is antagonized can be, for example,
10 IL-18, IL-12, or NK cells (*i.e.*, the pharmaceutical composition can comprise an IL-18
antagonist, an IL-12 antagonist or an NK cell antagonist, as described hereinbefore, a
corticosteroid and a pharmaceutically acceptable carrier).

In a preferred embodiment, a pharmaceutical composition of the invention
comprises an inhibitor of a caspase family protease, a corticosteroid and a pharmaceutically
acceptable carrier. Examples of inhibitors of caspase family proteases, and nonlimiting
15 exemplary dosages, are described hereinbefore. In a preferred embodiment, the inhibitor of
the caspase family protease is an ICE inhibitor.

In yet another embodiment, a pharmaceutical composition of the invention
comprises an IL-12 antagonist, a corticosteroid and a pharmaceutically acceptable carrier.
Examples of such IL-12 antagonists are described hereinbefore. In a preferred
20 embodiment, the IL-12 antagonist is an anti-IL-12 monoclonal antibody. In another
preferred embodiment, the IL-12 antagonist is a phosphodiesterase IV inhibitor. In yet
another preferred embodiment, the IL-12 antagonist is a beta-2 agonist.

In still another embodiment, a pharmaceutical composition of the invention
comprises an NK cell antagonist, a corticosteroid and a pharmaceutically acceptable carrier.
25 Examples of such NK cell antagonists are described hereinbefore. In a preferred
embodiment, the anti-NK cell antagonist is an anti-NK/NK-like cell antibody, preferably an
anti-asialo-GM1 antibody or an NK1.1 antibody.

As used herein the term "pharmaceutically acceptable carrier" is intended to include
any and all solvents, dispersion media, coatings, antibacterial and antifungal agents,
30 isotonic and absorption delaying agents, and the like, compatible with pharmaceutical
administration. The use of such media and agents for pharmaceutically active substances is
well known in the art. Except insofar as any conventional media or agent is incompatible
with the active compound, use thereof in the compositions is contemplated. Supplementary
active compounds can also be incorporated into the compositions.

35 A pharmaceutical composition of the invention is formulated to be compatible with
its intended route of administration. For example, solutions or suspensions used for
parenteral, intradermal, or subcutaneous application can include the following components:
a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols,

glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and

used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

The pharmaceutical compositions of the invention can be formulated for administration by a particular route of administration, such as oral administration, intravenous administration, ophthalmic administration, and the like.

In a preferred embodiment, a pharmaceutical composition of the invention is formulated for topical administration. Accordingly, an agent which antagonizes a target that regulates production of interferon- γ (IFN- γ) in the subject, a corticosteroid and a pharmaceutically acceptable carrier can be formulated into a cream, salve, ointment and the like suitable for application to the skin.

In another preferred embodiment, a pharmaceutical composition of the invention is formulated for administration by inhalation. Accordingly, an agent which antagonizes a target that regulates production of interferon- γ (IFN- γ) in the subject, a corticosteroid and a pharmaceutically acceptable carrier can be formulated into a nasal spray or an inhalant to allow for delivery of the therapeutic agents to the nasal or sinus passages or the lungs (*e.g.*, the bronchial passages) by inhalation.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references and published patents and patent applications cited throughout the application are hereby incorporated by reference.

EXAMPLE 1: Inhibition of ICE Activity in a Septic Shock Model Results in Steroid Responsiveness

In this example, the effect of inhibiting ICE activity on steroid responsiveness in septic shock was examined. A model of septic shock was induced in ICE-deficient (ICE -/-) and wild type (ICE +/+) mice, followed by treatment with a corticosteroid. The ICE -/- mice serve as a model of complete inhibition of ICE activity (see Li, P., *et al.* (1995) *Cell* 80:401-411 for further description of the ICE deficient mice). The responsiveness of the animals to corticosteroid treatment was determined by monitoring the levels of the inflammatory cytokine TNF α in the sera of the mice.

ICE-deficient and wild type mice first were sensitized with *Propionibacterium acnes* cell wall material (1 mg per mouse) to induce low grade inflammation and six days later were challenged with lipopolysaccharide (LPS) (1 µg per mouse in 0.1 ml of saline i.v.). Thirty minutes after LPS administration, the mice were treated with the corticosteroid dexamethasone (4 mg/kg per mouse in 0.5 ml 95% saline/0.5% ethanol, i.p.). Control mice were treated with vehicle alone. All mice were bled 90 minutes after LPS administration and the serum samples were analyzed for the presence of TNFα by standard ELISA.

The results are shown in Figure 1. Wild type and ICE deficient mice treated with vehicle alone had similar levels of serum TNF α . Treatment of wild type mice with dexamethasone did not significantly affect serum TNF α levels, demonstrating their resistance to steroid treatment in this septic shock model. In contrast, treatment of the ICE deficient mice with dexamethasone suppressed serum TNF α levels by 74% ($p<0.002$). These data indicate that inhibition of ICE activity reverses resistance to steroid treatment in a septic shock model.

EXAMPLE 2: Inhibition of ICE Activity in a Septic Shock Model Increases Steroid Sensitivity

In this example, the effect of inhibiting ICE activity on steroid sensitivity in septic shock was examined. The same LPS/*P. acnes* model of septic shock described in Example 1 was used, except that ICE deficient and wild type mice were pretreated with vehicle or a corticosteroid 15 minutes prior to challenge with LPS. The responsiveness of the animals to corticosteroid treatment again was determined by monitoring the levels of the inflammatory cytokine TNF α in the sera of the mice.

ICE-deficient and wild type mice first were sensitized with *Propionibacterium acnes* cell wall material (1 mg per mouse) to induce low grade inflammation and six days later were challenged with lipopolysaccharide (LPS) (1 µg per mouse in 0.1 ml of saline i.v.). Fifteen minutes prior to LPS challenge, the animals were treated with decreasing
5 amounts of the corticosteroid dexamethasone (0.05, 0.005 or 0.0005 mg/kg per mouse in 0.5 ml 95% saline/0.5% ethanol, i.p.). Control mice were treated with vehicle alone. All mice were bled 90 minutes after LPS administration and the serum samples were analyzed for the presence of TNFα by standard ELISA.

The results are shown in Figure 2. Both the wild type and the ICE deficient mice
10 exhibited responsiveness to pretreatment with 0.05 mg/kg of dexamethasone. In contrast, ICE deficient mice pretreated with only 0.005 or 0.0005 mg/kg dexamethasone exhibited 76% and 78% (p<0.005) lower serum TNFα levels, respectively, compared to the lack of TNFα suppression in the wild type animals similarly treated. These data indicate that
15 inhibition of ICE activity results in increased steroid sensitivity in a septic shock model, since 10-100 fold lower doses of dexamethasone were therapeutically effective in the ICE deficient animals as compared to the wild type animals.

EXAMPLE 3: A Phosphodiesterase IV Inhibitor Reduces IL-12 Production

20 In this example, the effect of a phosphodiesterase IV inhibitor, Rolipram, on LPS-induced IL-12 production was examined. B6 mice were pretreated with vehicle or Rolipram (30 mg/kg in 0.5 ml 0.1% methyl cellulose, i.p.) 15 minutes prior to challenge with LPS (10 µg/mouse, i.v.). Ninety minutes following LPS administration, the mice were bled and serum levels of IL-12 were determined by standard ELISA.

25 The results are shown in Figure 3. Mice pretreated with Rolipram had 70% lower serum IL-12 levels than mice pretreated with vehicle alone. These data indicate that phosphodiesterase IV inhibitors are effective for inhibiting the production of LPS-induced IL-12.

30 **EXAMPLE 4: Cleavage of IL-18 by Caspase Family Proteases**

The ability of various recombinant (*i.e.*, *E. coli* expressed) caspase family proteases to cleave precursor IL-18 (proIL-18) to mature IL-18 was tested in an *in vitro* proteolysis assay. Cleavage of poly(ADP-ribose) polymerase (PARP) was used as a positive control.
35 The results are summarized below in Table 1.

Table 1 Proteolysis of proIL-18 by Recombinant Caspases

<u>Caspase*</u>	<u>Concentration (µg/ml)</u>	<u>% Cleavage</u>	
		<u>proIL-18</u>	<u>PARP</u>
ICE (1)	1.25	100	99
ICH-2 (4)	5.00	82	93
ICE _{rel} III (5)	20.00	55	90
CPP32 (3)	5.00	0 ^a	100
Mch2 (6)	10.00	2	96
Mch3 (7)	5.00	32 ^b	97
ICH-1 (2)	75.00	5	98

* Caspases are numbered in parenthesis as recommended in Alnemri *et al.* (1996) *Cell* 87:171.

^a CPP32 (5 µg/ml) cleaved proIL-18 but generated a 12 kDa and a 10 kDa fragment instead of the expected 18 kDa fragment.

^b Unlike other caspases, the Mch3 precursor expressed in *E. coli* does not undergo autocatalysis to generate an active protease. Addition of ICE was required to initiate Mch3 autocatalysis and generate an active Mch3 protease. Partial cleavage of proIL-18 by Mch3 is mediated by the presence of ICE in the Mch3 preparation.

EXAMPLE 5: Treatment of Septic Shock

Patients who present in a clinical setting with septic shock (*e.g.*, in conjunction with infected abrasions, projectile wounds, or systemic bacteremias from other sources) are administered agent selected from an ICE inhibitor, a phosphodiesterase IV inhibitor (*e.g.*, rolipram, 30 mg/kg) and an anti-IL-12 monoclonal antibody, together with a corticosteroid (*e.g.*, high dose methylprednisolone, 1 gm/day, *i.v.*). The corticosteroid and the agent can be administered simultaneously, or alternatively, the agent can be administered before or after corticosteroid administration. Patients are also treated with appropriate antibiotic therapy.

EXAMPLE 6: Treatment of Transplant Rejection

Patients who are to receive a kidney transplant are administered an agent selected from ICE inhibitor, a phosphodiesterase IV inhibitor (*e.g.*, rolipram, 30 mg/kg) and an anti-IL-12 monoclonal antibody together with a corticosteroid (*e.g.*, oral prednisone, 25-75 mg/day). Treatment preferably is begun prior to receipt of the donated kidney (*e.g.*, drug administration may begin 24 hours prior to receipt of the donated kidney), with dosages to be repeated as needed (*e.g.*, every 12 hours). The corticosteroid and the agent can be

administered simultaneously, or alternatively, the agent can be administered before or after corticosteroid administration. Patients are also treated with additional immunosuppressive therapy (such as cyclosporin A treatment or OKT3 antibody treatment) so that immune rejection and inflammatory response are simultaneously suppressed.

5

EXAMPLE 7: Amelioration of the Steroid Rebound Effect

Patients with asthma, allergic rhinitis inflammation or rheumatoid arthritis who are undergoing treatment with a corticosteroid inhalant or with systemic corticosteroids, and
10 who are to enter a scheduled withdrawal from steroid treatment, are administered an agent selected from an ICE inhibitor, a phosphodiesterase IV inhibitor (*e.g.*, rolipram, 30 mg/kg) and an anti-IL-12 monoclonal antibody. Patients are preferably treated prior to the tapering or discontinuance of steroid treatment to ameliorate the steroid rebound effect that can result from cessation of steroid therapy. As needed, patients can be treated with additional
15 nonsteroidal anti-inflammatory agents.

EXAMPLE 8: Treatment of an Acute Episode of an Autoimmune Disease

Patients suffering from an acute flare-up of an autoimmune disease such as
20 inflammatory bowel disease (*e.g.*, ulcerative colitis or Crohn's disease) are administered an agent selected from ICE inhibitor, a phosphodiesterase IV inhibitor (*e.g.*, rolipram, 30 mg/kg) and an anti-IL-12 monoclonal antibody together with a corticosteroid (*e.g.*, oral prednisone, 25-75 mg/day). The corticosteroid and the agent can be administered simultaneously, or alternatively, the agent can be administered before or after corticosteroid
25 administration. Patients can also be treated with additional immunosuppressive therapy to control the acute flare up of the autoimmune disease.

EXAMPLE 9: Treatment of a Chronic Autoimmune Disease

30 Patients suffering from chronic autoimmune disease such as Crohn's disease are administered an agent selected from ICE inhibitor, a phosphodiesterase IV inhibitor (*e.g.*, rolipram, 30 mg/kg) and an anti-IL-12 monoclonal antibody together with a corticosteroid (*e.g.*, oral prednisone, 25-75 mg/day). The corticosteroid and the agent can be administered simultaneously, or alternatively, the agent can be administered before or after corticosteroid
35 administration. Patients can also be treated with additional immunosuppressive therapy to control the autoimmune disease.

EXAMPLE 10: Inhibition of IFN- γ Production by Elimination of NK Cells

In this example, shock was induced in mice by high dose LPS treatment (40 mg/kg LPS administered intravenously). The effect of depleting NK cells on the production of various cytokines in the mice and on mortality was examined by administering an anti-asialo-GM1 antibody (anti-ASGM1) intravenously 10 minutes prior to LPS administration. Control animals received Rabbit IgG. The effect of ASGM1 treatment on production of IL-1 β , TNF α and IFN- γ , as well as on mortality, is summarized below in Table 2:

10 Table 2

Treatment	# Mice	Cytokine Production (pg/ml)			Mortality (30 Hrs)
		IL-1 β	TNF α	IFN- γ	% Survival
LPS + Rabbit IgG (control)	6	1946 \pm 483	5857 \pm 1071	1663 \pm 811	0 (n=10)*
LPS + ASGM1 Antibodies	6	1647 \pm 482	5453 \pm 1103	363 \pm 108	90 (n=10)

* All animals died within 15 hrs.

These results indicate that elimination of NK cells by anti-asialo-GM1 antibody treatment reduces IFN- γ production and prolongs survival after LPS administration in high-dose LPS shock.

EXAMPLE 11: Effect of an ICE Inhibitor and Corticosteroid in Septic Shock Model

In this example, the LPS/*P. acnes* model of septic shock described in Examples 1 and 2 was used to examine the effect of an ICE inhibitor in combination with a corticosteroid. B6 mice were implanted with a 24 hour osmotic pump containing the ICE inhibitor acetyl-tyrosine-valine-alanine-aspartic acid-CHO (Ac-YVAD-CHO) (100 mg/kg), or a vehicle control, subcutaneously 18 hours before LPS injection. LPS was injected intravenously (0.01 μ g/mouse or 10 μ g/mouse) at time zero. All mice were injected with 5 mg/kg of dexamethasone intraperitoneally 30 minutes after LPS injection. The responsiveness of the animals to corticosteroid treatment was determined by monitoring the levels of the inflammatory cytokine TNF α , as well as interleukin-6 (IL-6) and interleukin-1 β (IL-1 β), in the sera of the mice. All mice were bled 90 minutes after LPS administration

and the serum samples were analyzed for the presence of TNF α , IL-6 and IL-1 β by standard methods.

The results are shown in Figures 4, 5 and 6, for TNF α , IL-6 and IL-1 β , respectively. The data demonstrate that mice treated with both the ICE inhibitor Ac-YVAD-CHO and dexamethasone had significantly lower serum levels of TNF α , IL-6 and IL-1 β , when either 0.01 μ g or 10 μ g of LPS was used to induce septic shock. As previously shown in Example 1, treatment of mice with dexamethasone alone did not significantly affect serum TNF α levels, demonstrating the resistance of the mice to steroid treatment alone in this septic shock model. In contrast, treatment of the mice with both dexamethasone and the ICE inhibitor suppressed serum TNF α levels by 96% ($p < 0.005$), in mice treated with 0.01 μ g of LPS, and by 86% ($p < 0.005$), in mice treated with 10 μ g of LPS. Moreover, serum IL-6 levels were reduced 95% ($p < 0.00005$) and 91% ($p < 0.00005$), respectively, and serum IL-1 β levels were reduced 94% ($p < 0.001$) and 92% ($p < 0.0002$), respectively. These data indicate that inhibition of ICE activity using an ICE inhibitor reverses resistance to steroid treatment in a septic shock model.

EXAMPLE 12: Synthesis of Hydroxamate ICE Inhibitors

3-Benzylloxycarbonylamino-4-oxo-5-phenylacetylaminooxy-pentanoic acid

Step A N-(Phenylmethoxy)-benzeneacetamide [(0.760 g, 3.15 mmol), prepared by the method of Hearn M.T.W. and Ward A.D. (*Aust. J. Chem.*, 1969;22:1731)] was taken up in 10 mL of CH₃CN and treated with dimethylamino-pyridine (DMAP) (50 mg) and di-tert-butyl dicarbonate (0.824 g, 3.78 mmol). The reaction was allowed to stir under Argon for 12 hours, then diluted with ethyl acetate (EtOAc) and washed with 3M K₂S₂O₅ (1 x 10 mL), NaHCO₃ (1 x 10 mL). The organic layer was dried over Na₂SO₄, filtered, and concentrated. Purification by chromatography (SiO₂, 9:1 hexane-EtOAc) afforded 0.910 g (84%) of 1,1-dimethylethyl(phenylacetyl)phenyl-methoxy)carbamate as a clear, viscous oil. ¹H NMR (400 MHz, DMSO-d₆): δ 7.41 [m, 5H], 7.32 [m, 2H], 7.24 [m, 3H], 4.90 [s, 2H], 4.09 [s, 2H], 1.48 [s, 9H]. IR (thin film) 3063, 3032, 2979, 2935, 2886, 1777, 1736, 1497, 1455, 1370, 1302 cm⁻¹. Mass Spectra (MS) (Chemical Ionization [CI] - NH₃) 342 (M⁺+H). Elemental Analysis: Calculated for C₂₀H₂₃NO₄·0.051 CH₂Cl₂: C, 69.66; H, 6.74; N, 4.05. Found: C, 69.66; H, 6.83; N, 3.99.

Step B 1,1-Dimethylethyl (phenylacetyl)(phenyl-methoxy)carbamate (810 mg, 2.37 mmol) was dissolved in 75 mL of dry THF and 90 mg of 5% Pd/BaSO₄ was added. The

reaction was treated with H₂ (20 psi) for 20 hours. The reaction was filtered through Celite and concentrated to obtain 588 mg (99%) of 1,1-dimethyl-ethyl hydroxy-(phenylacetyl)carbamate as an oil. No further purification was done.

¹H NMR (400 MHz, CDCl₃) : δ 8.22 [s, 1H], 7.31 [m, 5H], 4.24 [s, 2H], 1.55 [s, 9H].

Step C (S)-5-Bromo-4-oxo-3-[[[(phenylmethoxy)-carbonyl]amino]-pentanoic acid, 1,1-dimethylethyl ester [(297 mg, 0.742 mmol), prepared according to the procedure of Dolle R.E., et al., (*J. Med. Chem.*, 1994;37:563-4)], 1,1-dimethylethyl hydroxy(phenylacetyl)carbamate (187 mg, 0.742 mmol) and KF (104 mg, 1.85 mmol) were combined in 5 mL dimethylformamide (DMF) and allowed to stir under Ar for 12 hours. The reaction was diluted with EtOAc (15 mL) and washed with water (3 x 15 mL) and brine (1 x 15 mL). The organic layer was dried over Na₂SO₃ and concentrated. Purification by chromatography (SiO₂, 4:1 hexane-EtOAc) yielded 168 mg (40%) of [[[(1,1-dimethylethoxy)-carbonyl](phenylacetyl)amino]oxy]-4-oxo-3-[[[(phenylmethoxy)carbonyl]amino]-pentanoic acid, 1,1-dimethylethyl ester as a clear oil.

¹H NMR (300 MHz, CDCl₃) : δ 7.35 [m, 5H], 5.84 [d, J = 9.0 Hz, 1H], 4.79, [A of AB, J = 15.3 Hz, 1H], 4.70 [m, 1H], 4.57 [B of AB, J = 15.3 Hz, 1H], 4.10 [s, 2H], 3.09 [dd, J = 16.8, 4.6 Hz, 1H], 2.79 [dd, J = 16.8, 4.9 Hz, 1H], 1.52 [s, 9H], 1.39 [s, 9H]. IR (thin film) 3374, 2980, 2935, 1726 (br), 1499, 1370, 1298, 1150 cm⁻¹. MS (APCI, Methanol(MeOH)) 571.5 (M⁺+H).

Elemental Analysis:

Calculated for C₃₀H₃₈N₂O₉: C, 63.15; H, 6.71; N, 4.91.

Found: C, 62.76; H, 6.70; N, 4.69.

Step D 3-Benzylloxycarbonylamino-4-oxo-5-phenylacetylaminooxy-pentanoic acid, 1,1-dimethylethyl ester (208 mg, 0.365 mmol) was taken up in 3 mL of 1:1 trifluoroacetic acid (TFA) CH₂Cl₂ and allowed to stir for 2 hours. Reaction was diluted with acetonitrile (MeCN) (10 mL) and concentrated. The residue was stripped down from MeCN five times. Purification by chromatography (SiO₂, 90:9:1 CH₂Cl₂-acetone-formic acid) afforded 3-benzylloxycarbonylamino-4-oxo-5-phenylacetylaminooxy-pentanoic acid (51 mg, 34%) as a white foam

¹H NMR (300 MHz, CDCl₃) : δ 8.63 [s, 1H], 7.34 [broad (br) s, 10H], 5.48 [br d, J = 4 Hz, 1H], 5.08 [br dd, J = 16, 12 Hz, 2H], 4.23 [m, 1H], 3.97 [m, 2H], 3.58 [br s, 2H], 2.80 [m, 1H], 2.64 [m, 1H].

IR (KBr) 3305 (br), 2928, 1791, 1772, 1717, 1699, 1685, 1674, 1654, 1521, 1455 cm⁻¹.

MS (APCI, MeOH) 415 (M⁺+H).

Elemental Analysis:

Calculated for $C_{21}H_{22}N_2O_7 \cdot 0.106 CF_3CO_2H$: C, 59.73; H, 5.22; N, 6.57.

Found: C, 59.73; H, 5.46; N, 6.28.

- 5 The following were prepared from (S)-5-bromo-4-oxo-3-
[[[(phenylmethoxy)carbonyl]amino]-pentanoic acid, 1,1-dimethylethyl ester in the manner
described above, Step C, and Step D.

3-Benzyloxycarbonylamino-4-oxo-5-(2-oxo-pyrrolidin-1-yloxy)-pentanoic acid

- 10 Step A Prepared from 1-hydroxy-2-pyrrolidinone [Biswas A. and Miller M.J.
(*Heterocycles*, 1987;26:2849)] in the manner describe above, Step C to give 3-
benzyloxycarbonylamino-4-oxo-5-(2-oxo-pyrrolidin-1-yloxy)-pentanoic acid, 1,1-
dimethylethyl ester (74%).
 1H NMR (400 MHz, $CDCl_3$): δ 7.37 [m, 5H]; 5.88 [br d, J = 8.9
15 Hz]; 5.16 [A of AB, J = 12.2 Hz, 1H]; 5.11 [B of AB, J = 12.2
Hz, 1H]; 4.95 [A of AB, J = 17.1 Hz, 1H], 4.81 [B of AB, J =
17.1 Hz, 1H], 4.60 [m, 1H], 3.62 [m, 2H], 3.01 [dd, J = 17.1,
4.6 Hz, 1H], 2.75 [dd, J = 17.1, 4.8 Hz, 1H], 2.30 [t, J = 7.95 Hz, 2H], 1.99 [quint, J = 7.5
Hz, 2H], 1.41 [s, 9H]. IR (KBr) 3328 (br), 2976, 2932, 1717, 1701, 1522, 1256 cm^{-1} .
20 MS (APCI, MeOH) 421 ($M^+ + H$).

Elemental Analysis:

Calculated for $C_{21}H_{28}N_2O_7 \cdot 0.096 DMF$: C, 59.81; H, 6.76; N, 6.87.

Found: C, 59.56; H, 7.00; N, 6.52.

- 25 Step B: Prepared from 3-benzyloxycarbonylamino-4-oxo-5-(2-oxo-pyrrolidin-1-yloxy)-
pentanoic acid, 1,1-dimethylethyl ester in the manner described above, Step D to afford 3-
benzyloxycarbonylamino-4-oxo-5-(2-oxo-pyrrolidin-1-yloxy)-pentanoic acid (72%).
 1H NMR (400 MHz, $CDCl_3$): δ 8.55 [br s, 1H], 7.36 [m, 5H], 5.46
[br d, J = 9.4 Hz, 1H], 5.14 [A of AB, J = 5.2 Hz, 1H], 5.11
30 [B of AB, J = 5.2 Hz, 1H], 4.23 [m, 1H], 4.19 [A of AB, J = 13.3 Hz,
1H], 3.96 [B of AB, J = 13.3 Hz, 1H], 3.67 [m, 1H], 3.52 [dd, J = 15.1, 7.9 Hz, 1H], 2.84
[dd, J = 16.9, 8.2 Hz, 1H], 2.61 [dd, J = 16.9, 10.9 Hz, 1H], 2.42 [m, 2H], 2.11 [m, 2H]. IR
(KBr) 3408 (br), 2926, 1791, 1717, 1700, 1540, 1268, 1054 cm^{-1} . MS (APCI, MeOH) 365
($M^+ + H$).
35 Analysis calculated for $C_{17}H_{20}N_2O_7 \cdot 0.32 C_3H_7OC_3H_7$: C, 57.27; H, 6.24; N, 7.04.
Found: C, 57.27; H, 6.24; N, 6.74.

3-Benzylloxycarbonylamino-5-(3,5-dioxo-10-oxa-4-aza-tricyclo[5.2.1.0^{2,6}]dec-8-en-4-yloxy)-4-oxo-pentanoic acid

- Step A Prepared from 3a,4,7,7a-tetrahydro-2-hydroxy-4,7-epoxy-1H-isoindole-1,3(2H)-dione [Narita M., Teramoto T, Okawara M (*Bull. Chem. Soc. Jap.*, 1971;44:1084)] in the manner described above, Step C, to afford 3-benzylloxycarbonylamino-5-(3,5-dioxo-10-oxa-4-aza-tricyclo[5.2.1.0^{2,6}]dec-8-en-4-yloxy)-4-oxo-pentanoic acid, 1,1-dimethylethyl ester (64%). ¹H NMR (400 MHz, DMSO-d₆): δ 7.84 [d, J = 8.2 Hz, 1H], 7.34 [m, 5H], 6.54 [s, 2H], 5.16 [s, 2H]; 5.07 [A of AB, J = 12.5 Hz, 1H], 5.03 [B of AB, J = 12.5 Hz, 1H], 4.93 [A of AB, J = 16.2 Hz, 1H], 4.87 [B of AB, J = 16.2 Hz, 1H], 4.52 [m, 1H], 2.87 [s, 2H], 2.73 [dd, J = 16.2, 5.8 Hz, 1H], 2.50 [obscured by dimethyl-sulfoxide (DMSO) resonance], 1.37 [s, 9H]. IR (KBr) 3421, 2979, 2930, 1790, 1726, 1520, 1368 cm⁻¹. MS (APCI, MeOH) 445 (M⁺-C₄H₈). Analysis calculated for C₂₅H₂₈N₂O₉: C, 59.65; H, 5.70; N, 5.35. Found: C, 59.99; H, 5.64; N, 5.60.

- Step B Prepared from 3-benzylloxycarbonylamino-5-(3,5-dioxo-10-oxa-4-aza-tricyclo[5.2.1.0^{2,6}]dec-8-en-4-yloxy)-4-oxo-pentanoic acid, 1,1-dimethylethyl ester in the manner described above, Step C to give 3-benzyloxy-carbonylamino-4-oxo-5-phenylacetylaminooxy-pentanoic acid (78%). IR (thin film) 3360, 1789, 1723, 1530, 1220 cm⁻¹. MS (APCI, MeOH) 445 (M⁺+H).
Elemental Analysis:
Calculated for C₂₁H₂₀N₂O₉·0.194 CF₃CO₂H: C, 55.06; H, 4.36; N, 5.96.
Found: C, 55.06; H, 4.58; N, 5.99.

- 3-Benzylloxycarbonylamino-5-(2-oxo-2,3-dihydro-indol-1-yloxy)-4-oxo-pentanoic acid
Prepared from 1-hydroxyoxindole [Kende A.S. and Thurston J. (*Synthetic Communications*, 1990;20:2133-8)] to give 3-benzylloxycarbonylamino-4-oxo-5-(2-oxo-2,3-dihydro-indol-1-yloxy)-pentanoic acid (24%), mp 58-70°C (decomposes).
Elemental Analysis:
Calculated for C₂₁H₂₀N₂O₇: C, 61.16; H, 4.89; N, 6.79.
Found: C, 60.84; H, 4.72; N, 6.46.

3-Benzylloxycarbonylamino-5-(7-methoxycarbonylmethyl-2-oxo-octahydro-indol-1-yloxy)-4-oxo-pentanoic acid

- Step A Hydroxylamine hydrochloride (200 mmol, 13.8 g) was dissolved in pyridine (200 mmol, 16 mL) and methanol (10 mL), and this solution was added to a mixture of cis-2-oxo-1,3-cyclo-hexanediacetic acid, dimethyl ester [(35 mmol, 8.5 g) prepared following the procedure of Grieco P.A., Noguez J.A., Masaki Y., Hiroi K., Nishizawa M.,

Rosowsky A., Oppenheim S., Lazarus H. *J. Med. Chem.*, 1977;20:71] in 200 mL of MeOH. To this solution NaCNBH_4 (30 mmol, 1.9 g) was added in portions over about 1 hour and the resulting solution was stirred at room temperature for 4 days. The reaction mixture was then concentrated to dryness, redissolved in 500 mL ethyl acetate, and washed 3 x 50 mL saturated NaCl, dried with Na_2SO_4 , filtered, and concentrated to yield a crude solid, which was mostly desired product and pyridine. Crude octahydro-1-hydroxy-2-oxo-1H-indole-7-acetic acid, methyl ester was recrystallized from EtOAc to yield 4.05 g (51%) of a white solid.

$^1\text{H-NMR}$: 9.26 [1H, s], 3.64 [1H, dd], 3.59 [3H, s], 2.65 [1H, dd], 2.49 [1H, dd], 2.34 [1H, dd], 2.18 [1H, m], 2.04 [1H, m], 1.79, 1H, d], 1.62 [1H, m], 1.60 [1H, s-br], 1.42 [1H, m], 1.25 [2H, m], 1.06 [1H, m]. MS (CI, NH_3) 228 ($\text{M}^+ + \text{H}$).

Step B Prepared from octahydro-1-hydroxy-2-oxo-1H-indole-7-acetic acid, methyl ester in the manner described above, Step C, to afford 3-benzyloxycarbonylamino-5-(7-methoxycarbonylmethyl-2-oxo-octahydro-indol-1-yloxy)-4-oxo-pentanoic acid, 1,1-trimethylethyl ester as a glassy oil (45%).

$^1\text{H NMR}$ (400 MHz, DMSO-d_6 , 1:1 mix of diastereomers): δ 7.85 [d, $J = 5.8 \text{ Hz}$, 0.5H], 7.83 [d, $J = 5.8 \text{ Hz}$, 0.5H], 7.35 [m, 5H], 5.06 [s, 2H], 4.94 [A of AB, $J = 16.9 \text{ Hz}$, 0.25H], 4.87 [A of AB, $J = 17.6 \text{ Hz}$, 0.25H], 4.82 [B of AB, $J = 17.6 \text{ Hz}$, 0.25H], 4.74 [B of AB, $J = 16.9 \text{ Hz}$, 0.25H], 4.23 [m, 1H], 3.82 [m, 0.5H], 3.79 [m, 0.5H], 3.57 [s, 1.5H]; 3.57 [s, 1.5H], 2.72 [m, 0.5H], 2.70 [m, 0.5H], 2.52 [m, obscured by DMSO], 2.39 [m, 2H], 2.22 [br m, 1H], 2.10 [br m, 1H], 1.88 [br s, 0.5H], 1.84 [br s, 0.5H], 1.61 [m, 2H], 1.42 [m, 1H], 1.36 [s, 9H], 1.25 [m, 2H], 1.06 [m, 1H]. IR (thin film) 3418, 3344, 3017, 2979, 2934, 2860, 1725, 1506 cm^{-1} . MS (APCI, MeOH) 547.6 ($\text{M}^+ + \text{H}$).

Step C Prepared from 3-benzyloxycarbonylamino-5-(7-methoxycarbonylmethyl-2-oxo-octahydro-indol-1-yloxy)-4-oxo-pentanoic acid, 1,1-trimethylethyl ester in the manner described above, step D to afford 3-benzyloxy-carbonylamino-5-(7-methoxycarbonylmethyl-2-oxo-octahydro-indol-1-yloxy)-4-oxo-pentanoic acid (45%), mp 55-58°C.

$^1\text{H NMR}$ (400 MHz, DMSO-d_6 , 1:1 mix of diastereomers): δ 12.4 [s, 1H], 7.84 [m, 1H], 7.35 [m, 5H], 5.05 [s, 2H], 4.86 [m, 2H], 4.45 [m, 1H], 3.83 [m, 0.5H], 2.79 [m, 0.5H], 3.59 [s, 1.5H], 3.58 [s, 0.5H], 2.57 [m, obscured by DMSO], 2.41 [complex m, 4H], 2.20 [m, 2H], 1.88 [m, 1H], 1.62 [m, 2H], 1.43 [m, 2H], 1.23 [m, 2H], 1.05 [m, 1H]. IR (KBr) 3337, 2931, 1790, 1726, 1538 cm^{-1} . MS (ES, NH_4OH) 489.5 ($\text{M}^+ - \text{H}$).

Elemental Analysis:

Calculated for $C_{24}H_{30}N_2O_9$: C, 58.77; H, 6.16; N, 5.71.

Found: C, 59.19; H, 6.40; N, 5.34.

3-Benzylloxycarbonylamino-4-oxo-5-(2-oxo-octahydro-indol-1-yloxy)-pentanoic acid

- 5 Step A Ethyl 2-cyclohexanoneacetate (4.28 g, 23.3 mmol) and O-benzyl hydroxylamine hydrochloride were combined in 100 mL of ethanol (EtOH) and 2.59 g (25.6 mmol, 3.55 mL) of triethyl amine(Et_3N) was added. The reaction was stirred at room temperature for 12 hours at which point it was concentrated in vacuo. The residue was taken up in EtOAc and washed with 1N HCl (2 x 20 mL), saturated $NaHCO_3$ (1 x 20 mL), dried over Na_2SO_4 ,
10 filtered, and concentrated. Purification by chromatography (SiO_2 , 90:1 hexanes-EtOAc) afforded (2-benzyl oxyimino-cyclohexyl)-acetic acid ethyl ester (4.76 g, 72%) as a mixture of oxime isomers.

- 1H NMR (400 MHz, $CDCl_3$ 7:1 mixture of oxime isomers): δ 7.32 [complex m, 5H], 5.05 [s, 0.25 H], 5.02 [s, 1.H], 4.05 [q, J = 7.2 Hz, 2H], 3.20 [m, 1H], 2.73 [complex m, 2H], 2.46 [d, J = 8.0 Hz, 0.125H], 2.21 [dd, J = 15.4, 6.3 Hz, 0.875H], 1.92 [m, 1H], 1.79 [complex m, 3H], 1.43 [m, 1H], 1.38 [complex m, 2H], 1.22 [t, J = 7.2 Hz, 3H]. IR (thin film) 2931, 1735, 1638, 1451 cm^{-1} . MS (CI, NH_3) 290 ($M^+ + H$).

Calculated for $C_{17}H_{23}N_1O_3$: C, 70.56; H, 8.01; N, 4.84.

- 20 Found: C, 70.47; H, 7.92; N, 4.78.

- Step B (2-Benzyl oxyimino-cyclohexyl)-acetic acid ethyl ester (4.66 g, 16.1 mmol) was taken up in 15 mL of acetic acid (AcOH) and $NaBH_3CN$ and stirred for 72 hours. Reaction was poured into $NaHCO_3$ and extracted into EtOAc (3 x 30 mL). The combined organic
25 layers were washed once with brine, dried over Na_2SO_4 , filtered, and concentrated. The clear oil was dissolved in 50 mL of MeOH and K_2CO_3 (5.55 g, 40.2 mmol) was added and the reaction stirred for 12 hours. The reaction was concentrated, the residue taken up in $CHCl_3$, filtered, and concentrated. Purification by chromatography (SiO_2 , 4:1 hexanes/EtOAc) afforded 1.72 g (43%) of cis-(2-benzyl oxyamino-cyclohexyl)-acetic acid
30 ethyl ester and 0.441 g (11%) of trans-(2-benzyl oxyamino-cyclohexyl)-acetic acid ethyl ester.

Data for cis isomer:

- 1H NMR (400 MHz, $CDCl_3$): δ 7.44 [complex m, 2H], 7.37 [complex m, 3H], 5.05 [A of AB, J = 10.4 Hz, 1H], 4.94 [B of AB, J = 10.4 Hz, 1H], 3.47 [dd, J = 10.6, 5.3 Hz, 1H], 2.33 [dd, J = 16.4 Hz, 1H], 2.20 [m, 1H], 2.08 [dd, J = 16.4, 4.6 Hz, 1H], 1.74 [complex m, 2H], 1.60 [m, 1H], 1.32 [complex m, 5H]. IR (solution, $CHCl_3$) 3031, 2932, 2856, 1717, 1453 cm^{-1} . MS (CI, NH_3) 246 ($M^+ + H$).

Data for trans isomer: mp 79-82°C.

Elemental Analysis:

Calculated for $C_{15}H_{19}N_1O_2$: C, 73.44; H, 7.81; N, 5.71.

Found: C, 73.38; H, 7.89; N, 5.63.

5

Step C Prepared from cis-(2-benzyloxyamino-cyclohexyl)-acetic acid ethyl ester in the manner described above, Step B to give cis-1-hydroxy-octahydro-indol-2-one (85%), mp 85-86°C.

1H NMR (400 MHz, $CDCl_3$): δ 9.86 [br s, 1H], 3.75 [dd, J = 10.4, 4.8 Hz, 1H], 2.41 [dd, J = 16.1, 7.7 Hz, 1H], 2.33 [m, 1H], 1.97 [m, 1H], 1.71 [complex m, 2H], 1.54 [m, 1H], 1.44 [complex m, 2H], 1.31 [complex m, 2H]. IR (KBr) 3037, 2936, 2856, 2710, 1690, 1659, 1548 cm^{-1} . MS (CI, NH_3) 156 ($M^+ + H$).

Elemental Analysis:

15 Calculated for $C_8H_{13}N_1O_2$: C, 61.91; H, 8.44; N, 9.03.

Found: C, 61.94; H, 8.49; N, 8.96.

Step D Prepared from cis-1-hydroxy-octahydro-indol-2-one in the manner described above step C to afford 3-benzyloxycarbonylamino-4-oxo-5-(2-oxo-octahydro-indol-1-yloxy)-pentanoic acid, 1,1 dimethylethyl ester (41%). IR (thin film) 2933, 1723, 1516, 1367 cm^{-1} .

20 Elemental Analysis:

Calculated for $C_{25}H_{34}N_2O_7$: C, 63.28; H, 7.22; N, 5.90.

Found: C, 63.03; H, 7.36; N, 5.65.

25 Step E Prepared from 3-benzyloxycarbonylamino-4-oxo-5-(2-oxo-octahydro-indol-1-yloxy)-pentanoic acid, 1,1- dimethylethyl ester in the manner described above, Step D to afford 3-benzyloxycarbonylamino-4-oxo-5-(2-oxo-octahydro-indol-1-yloxy)-pentanoic acid (72%). IR (KBr) 3352 (br), 2935, 2869, 1789, 1704, 1535 cm^{-1} . MS (APCI, MeOH) 419.5 ($M^+ + H$).

30 Elemental Analysis:

Calculated for $C_{21}H_{26}N_2O_7 \cdot 0.12 H_2O \cdot 0.322 CH_2Cl_2$:

C, 57.17; H, 6.05; N, 6.26.

Found: C, 57.17; H, 6.05; N, 5.89.

35 The following were prepared from 5-bromo-3-[2-(2-benzyloxycarbonylamino-3-methyl-butyrylamino)-propionylamino]-4-oxo-pentanoic acid, 1,1-dimethyl ester [Dolle R.E., et al. (*J. Med. Chem.*, 1994;37:563-4)] in the manner described above, Step C and Step D.

3-[2-(2-Benzylloxycarbonylamino-3-methyl-butylamino)-propionylamino]-5-(7-methoxycarbonylmethyl-2-oxo-octahydro-indol-1-yl)-4-oxo-pentanoic acid

- Prepared from octahydro-1-hydroxy-2-oxo-1H-indole-7-acetic acid, methyl ester (65%), mp 162-167°C, dec. Elemental Analysis:
Calculated for $C_{29}H_{34}N_4O_9 \cdot 0.75 H_2O$ (596.127): C, 58.43; H, 6.00; N, 9.40.
Found: C, 58.40; H, 5.68; N, 9.19.

3-[2-(2-benzylloxycarbonylamino-3-methyl-butylamino)-propionylamino]-4-oxo-5-(2-oxo-2,3-dihydro-indol-1-yl)-pentanoic acid

- Prepared from 1-hydroxyoxindole [Kende A.S. and Thurston J. (*Synthetic Communications*, 1990;20:2133-8)] to afford 3-[2-(2-benzylloxycarbonylamino-3-methyl-butylamino)-propionylamino]-4-oxo-5-(2-oxo-2,3-dihydro-indol-1-yl)-pentanoic acid (67%), mp 162-167°C, dec.
Elemental Analysis:
Calculated for $C_{29}H_{34}N_4O_9 \cdot 0.75 H_2O$ (596.127):
C, 58.43; H, 6.00; N, 9.40.
Found: C, 58.40; H, 5.68; N, 9.19.

- Other compounds were prepared using automated parallel synthesis, as follows:

- To a 7-mL screw top glass vial containing 17 mg (0.3 mmol, 3 eq) of potassium fluoride was added 500 μ L (0.1 mmol, 1 eq) of a 0.2 M solution of the appropriate hydroxamate in DMF. The reaction vial was agitated for a few minutes and the potassium fluoride did not completely dissolve. At this point, 500 μ L (0.1 mmol, 1 eq) of 0.2 M solution of (S)-5-bromo-4-oxo-3-[[[(phenylmethoxy)carbonyl]amino]-pentanoic acid, 1,1-dimethylethyl ester in DMF. The vials were capped and the rack of 30 to 40 vials were placed atop a circular agitator for 12 hours.

- The reaction mixtures were diluted with 2 mL of ethyl acetate followed by 2 mL of deionized water. Two milliliters of liquid was withdrawn from the middle of the vial and injected rapidly back in twice. The vials were allowed to sit for 30 minutes and the organic layer was withdrawn from the upper half of the vial. Twice more, 2 mL of ethyl acetate was added, mixed, and separated. The combined organic layers were evaporated under a steady stream of nitrogen overnight.

- The crude residue from the reactions were dissolved in 3 to 4 mL of 40% TFA in methylene chloride. The vials were agitated to ensure complete dissolution in a fume hood without caps. After 2 hours the vials were again placed under a steady stream of nitrogen overnight.

The crude reaction mixture was taken up in 1 mL of chloroform (MeOH was sometimes added to complete dissolution). The solutions were applied to 500- μ preparative silica gel TLC plates and then eluted with 20% acetone in methylene chloride with 1% to 2% acetic acid. The product bands were visualized by UV absorption, scraped
5 from the plate, and the silica gel washed with methanol into a tared vial. The vials were placed under a stream of nitrogen overnight. The weighed purified products were then diluted to 10 mM in 25% methanol in chloroform and aliquoted to plates for both analytical analysis and biological evaluation. The solutions were allowed to evaporate in the fume
10 hood over 72 hours.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following
15 claims.

CLAIMS

We claim:

- 5 1. A method for modulating responsiveness to a corticosteroid in a subject, comprising administering to the subject:
an agent which antagonizes a target that regulates production of interferon- γ (IFN- γ) in the subject, the agent being administered at a dosage and by a route sufficient to inhibit production of IFN- γ in the subject; and
10 a corticosteroid,
such that responsiveness of the subject to the corticosteroid is modulated as compared to when a corticosteroid alone is administered to the subject.
2. The method of claim 1, wherein the agent is an IL-18 antagonist, the agent being
15 administered at a dosage and by a route sufficient to inhibit IL-18 activity in the subject.
3. The method of claim 2, wherein the agent is an inhibitor of a caspase family protease.
- 20 4. The method of claim 3, wherein the agent is an ICE inhibitor.
5. The method of claim 2, wherein the agent is an antibody, antibody fragment, or engineered binding protein that binds IL-18 or an IL-18 receptor.
- 25 6. The method of claim 1, wherein the agent is an interleukin-12 (IL-12) antagonist, the agent being administered at a dosage and by a route sufficient to inhibit IL-12 activity in the subject.
7. The method of claim 6, wherein the agent is an antibody, antibody fragment, or
30 engineered binding protein that binds IL-12 or IL-12 receptor.
8. The method of claim 6, wherein the agent stimulates cyclic AMP production in cells that produce IL-12.
- 35 9. The method of claim 8, wherein the agent is a phosphodiesterase IV inhibitor.

10. The method of claim 9, wherein the phosphodiesterase IV inhibitor is selected from the group consisting of 4-arylpyrrolidinones, rolipram, denbufylline, tibenelast, nitraquazone, CP-80633, quinazolinediones and CP-77059.
- 5 11. The method of claim 8, wherein the agent is a beta-2 agonist.
12. The method of claim 11, wherein the beta-2 agonist is selected from the group consisting of salmeterol, fenoterol and isoproterenol.
- 10 13. The method of claim 6, wherein the agent is a STAT4 inhibitor.
14. The method of claim 1, wherein the agent is an NK cell antagonist.
- 15 15. The method of claim 14, wherein the agent is an anti-NK/NK-like cell antibody.
16. The method of claim 15, wherein the antibody is an anti-asialo-GM1 antibody or an NK1.1 antibody.
17. The method of claim 1, wherein the corticosteroid is selected from the group
20 consisting of cortisone, hydrocortisone, beclomethasone, flunisolide, prednisone, prednisolone, methylprednisolone, triamcinolone, deflazacort, betamethasone and dexamethasone.
18. The method of claim 1, wherein the subject is suffering from septic shock.
- 25 19. The method of claim 1, wherein the subject is suffering from Crohn's disease.
20. The method of claim 1, wherein the subject is suffering from asthma.
- 30 21. The method of claim 1, wherein the subject is suffering from graft versus host disease or transplant rejection.
22. The method of claim 1, wherein the subject is suffering from an autoimmune disease or disorder.
- 35 23. The method of claim 1, wherein the subject is suffering from an immunoinflammatory disease or disorder selected from the group consisting of asthma, adult respiratory distress syndrome, systemic lupus erythematosus, inflammatory bowel

- disease, Crohn's disease, ulcerative colitis, multiple sclerosis, insulin-dependent diabetes mellitus, autoimmune arthritis, rheumatoid arthritis, juvenile rheumatoid arthritis, psoriatic arthritis, inflammatory pulmonary syndrome, pemphigus vulgaris, idiopathic thrombocytopenic purpura, autoimmune meningitis, myasthenia gravis, autoimmune thyroiditis, dermatitis, atopic dermatitis, eczematous dermatitis, psoriasis, Sjögren's Syndrome, keratoconjunctivitis sicca secondary to Sjögren's Syndrome, alopecia areata, allergic responses due to arthropod bite reactions, aphthous ulcer, iritis, conjunctivitis, keratoconjunctivitis, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, drug eruptions, Stevens-Johnson syndrome, leprosy reversal reactions, erythema nodosum
- 10 leprosum, autoimmune uveitis, allergic encephalomyelitis, aplastic anemia, pure red cell anemia, idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active hepatitis, Graves ophthalmopathy, primary biliary cirrhosis, uveitis posterior and interstitial lung fibrosis.
- 15 24. The method of claim 1, wherein the subject is suffering from an acute inflammatory disorder.
25. The method of claim 1, wherein the subject is suffering from a chronic inflammatory disorder.
- 20 26. The method of claim 1, wherein steroid resistance in the subject is reversed, as compared to when a corticosteroid alone is administered to the subject
27. The method of claim 1, wherein steroid sensitivity in the subject is increased, as
- 25 compared to when a corticosteroid alone is administered to the subject.
28. The method of claim 1, wherein the corticosteroid is administered to the subject according to a schedule that reduces the dosage of the corticosteroid over time and the method ameliorates a steroid rebound effect associated with administration of reduced
- 30 dosages of the corticosteroid.
29. A method for modulating responsiveness to corticosteroids in a subject, comprising administering to the subject:
- 35 an agent that is an inhibitor of a caspase family protease; and
a corticosteroid,
- such that responsiveness of the subject to the corticosteroid is modulated as compared to when a corticosteroid alone is administered to the subject.

30. The method of claim 29, wherein the agent is an ICE inhibitor.
31. The method of claim 29, wherein the corticosteroid is selected from the group consisting of cortisone, hydrocortisone, beclomethasone, flunisolide, prednisone, prednisolone, methylprednisolone, triamcinolone, deflazacort, betamethasone and dexamethasone.
32. The method of claim 29, wherein the subject is suffering from septic shock.
33. The method of claim 29, wherein the subject is suffering from Crohn's disease.
34. The method of claim 29, wherein the subject is suffering from asthma.
35. The method of claim 29, wherein the subject is suffering from graft versus host disease or transplant rejection.
36. The method of claim 29, wherein the subject is suffering from an autoimmune disease or disorder.
37. The method of claim 29, wherein the subject is suffering from an immunoinflammatory disease or disorder selected from the group consisting of asthma, adult respiratory distress syndrome, systemic lupus erythematosus, inflammatory bowel disease, Crohn's disease, ulcerative colitis, multiple sclerosis, insulin-dependent diabetes mellitus, autoimmune arthritis, rheumatoid arthritis, juvenile rheumatoid arthritis, psoriatic arthritis, inflammatory pulmonary syndrome, pemphigus vulgaris, idiopathic thrombocytopenic purpura, autoimmune meningitis, myasthenia gravis, autoimmune thyroiditis, dermatitis, atopic dermatitis, eczematous dermatitis, psoriasis, Sjögren's Syndrome, keratoconjunctivitis sicca secondary to Sjögren's Syndrome, alopecia areata, allergic responses due to arthropod bite reactions, aphthous ulcer, iritis, conjunctivitis, keratoconjunctivitis, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, drug eruptions, Stevens-Johnson syndrome, leprosy reversal reactions, erythema nodosum leprosum, autoimmune uveitis, allergic encephalomyelitis, aplastic anemia, pure red cell anemia, idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active hepatitis, Graves ophthalmopathy, primary biliary cirrhosis, uveitis posterior and interstitial lung fibrosis.
38. The method of claim 29, wherein the subject is suffering from an acute inflammatory disorder.

39. The method of claim 29, wherein the subject is suffering from a chronic inflammatory disorder.

5 40. The method of claim 39, wherein steroid resistance in the subject is reversed, as compared to when a corticosteroid alone is administered to the subject

41. The method of claim 39, wherein steroid sensitivity in the subject is increased, as compared to when a corticosteroid alone is administered to the subject.

10

42. The method of claim 39, wherein the corticosteroid is administered to the subject according to a schedule that reduces the dosage of the corticosteroid over time and the method ameliorates a steroid rebound effect associated with administration of reduced dosages of the corticosteroid.

15

43. A method for modulating responsiveness to corticosteroids in a subject, comprising administering to the subject:

an agent that is an antagonist of interleukin-12 (IL-12), the agent being administered at a dosage and by a route sufficient to inhibit IL-12 activity in the subject; and

20

a corticosteroid,
such that responsiveness of the subject to the corticosteroid is modulated as compared to when a corticosteroid alone is administered to the subject.

44. The method of claim 43, wherein the agent is an antibody, antibody fragment, or
25 engineered binding protein that binds IL-12 or IL-12 receptor.

45. The method of claim 44, wherein the agent is an anti-IL-12 monoclonal antibody.

46. The method of claim 43, wherein the corticosteroid is selected from the group
30 consisting of cortisone, hydrocortisone, beclomethasone, flunisolide, prednisone, prednisolone, methylprednisolone, triamcinolone, deflazacort, betamethasone and dexamethasone.

47. The method of claim 43, wherein the subject is suffering from septic shock.

35

48. The method of claim 43, wherein the subject is suffering from Crohn's disease.

49. The method of claim 43, wherein the subject is suffering from asthma.

50. The method of claim 43, wherein the subject is suffering from graft versus host disease or transplant rejection.

5 51. The method of claim 43, wherein the subject is suffering from an autoimmune disease or disorder.

52. The method of claim 43, wherein the subject is suffering from an immunoinflammatory disease or disorder selected from the group consisting of asthma,
10 adult respiratory distress syndrome, systemic lupus erythematosus, inflammatory bowel disease, Crohn's disease, ulcerative colitis, multiple sclerosis, insulin-dependent diabetes mellitus, autoimmune arthritis, rheumatoid arthritis, juvenile rheumatoid arthritis, psoriatic arthritis, inflammatory pulmonary syndrome, pemphigus vulgaris, idiopathic thrombocytopenic purpura, autoimmune meningitis, myasthenia gravis, autoimmune
15 thyroiditis, dermatitis, atopic dermatitis, eczematous dermatitis, psoriasis, Sjögren's Syndrome, keratoconjunctivitis sicca secondary to Sjögren's Syndrome, alopecia areata, allergic responses due to arthropod bite reactions, aphthous ulcer, iritis, conjunctivitis, keratoconjunctivitis, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, drug eruptions, Stevens-Johnson syndrome, leprosy reversal reactions, erythema nodosum
20 leprosum, autoimmune uveitis, allergic encephalomyelitis, aplastic anemia, pure red cell anemia, idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active hepatitis, Graves ophthalmopathy, primary biliary cirrhosis, uveitis posterior and interstitial lung fibrosis.

25 53. The method of claim 43, wherein the subject is suffering from an acute inflammatory disorder.

54. The method of claim 43, wherein the subject is suffering from a chronic inflammatory disorder.

30

55. The method of claim 43, wherein steroid resistance in the subject is reversed, as compared to when a corticosteroid alone is administered to the subject

56. The method of claim 43, wherein steroid sensitivity in the subject is increased, as
35 compared to when a corticosteroid alone is administered to the subject.

57. The method of claim 43, wherein the corticosteroid is administered to the subject according to a schedule that reduces the dosage of the corticosteroid over time and the

method ameliorates a steroid rebound effect associated with administration of reduced dosages of the corticosteroid.

58. A method for modulating responsiveness to corticosteroids in a subject, comprising
5 administering to the subject:
an NK cell antagonist, the NK cell antagonist being administered at a dosage and by
a route sufficient to inhibit IFN- γ activity in the subject; and
a corticosteroid,
such that responsiveness of the subject to the corticosteroid is modulated as compared to
10 when a corticosteroid alone is administered to the subject.

59. The method of claim 58, wherein the agent is an antibody, antibody fragment, or
engineered binding protein that binds to an NK/NK-like cell surface marker.
- 15 60. The method of claim 59, wherein the agent is an anti-NK/NK-like cell antibody.
61. The method of claim 60, wherein the antibody is an anti-asialo-GM1 antibody or an
NK1.1 antibody.
- 20 62. The method of claim 58, wherein the subject is suffering from septic shock.
63. The method of claim 58, wherein the subject is suffering from Crohn's disease.
64. The method of claim 58, wherein the subject is suffering from asthma.
- 25 65. The method of claim 58, wherein the subject is suffering from graft versus host
disease or transplant rejection.
66. The method of claim 58, wherein the subject is suffering from an autoimmune
30 disease or disorder.
67. The method of claim 58, wherein the subject is suffering from an
immunoinflammatory disease or disorder selected from the group consisting of asthma,
adult respiratory distress syndrome, systemic lupus erythematosus, inflammatory bowel
35 disease, Crohn's disease, ulcerative colitis, multiple sclerosis, insulin-dependent diabetes
mellitus, autoimmune arthritis, rheumatoid arthritis, juvenile rheumatoid arthritis, psoriatic
arthritis, inflammatory pulmonary syndrome, pemphigus vulgaris, idiopathic
thrombocytopenic purpura, autoimmune meningitis, myasthenia gravis, autoimmune

thyroiditis, dermatitis, atopic dermatitis, eczematous dermatitis, psoriasis, Sjögren's Syndrome, keratoconjunctivitis sicca secondary to Sjögren's Syndrome, alopecia areata, allergic responses due to arthropod bite reactions, aphthous ulcer, iritis, conjunctivitis, keratoconjunctivitis, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, drug
5 eruptions, Stevens-Johnson syndrome, leprosy reversal reactions, erythema nodosum leprosum, autoimmune uveitis, allergic encephalomyelitis, aplastic anemia, pure red cell anemia, idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active hepatitis, Graves ophthalmopathy, primary biliary cirrhosis, uveitis posterior and interstitial lung fibrosis.

10

68. The method of claim 58, wherein the subject is suffering from an acute inflammatory disorder.

15

69. The method of claim 58, wherein the subject is suffering from a chronic inflammatory disorder.

20

70. The method of claim 58, wherein steroid resistance in the subject is reversed, as compared to when a corticosteroid alone is administered to the subject

71. The method of claim 58, wherein steroid sensitivity in the subject is increased, as compared to when a corticosteroid alone is administered to the subject.

25

72. The method of claim 58, wherein the corticosteroid is administered to the subject according to a schedule that reduces the dosage of the corticosteroid over time and the method ameliorates a steroid rebound effect associated with administration of reduced dosages of the corticosteroid.

30

73. A method for modulating responsiveness to a corticosteroid in a subject, comprising:

selecting a subject in need of modulation of responsiveness to a corticosteroid; and
administering to the subject an agent which antagonizes a target that regulates
production of interferon- γ (IFN- γ) in the subject, the agent being administered at a dosage
and by a route sufficient to inhibit production of IFN- γ in the subject,
such that responsiveness of the subject to a corticosteroid is modulated as compared to
35 when a corticosteroid alone is administered to the subject.

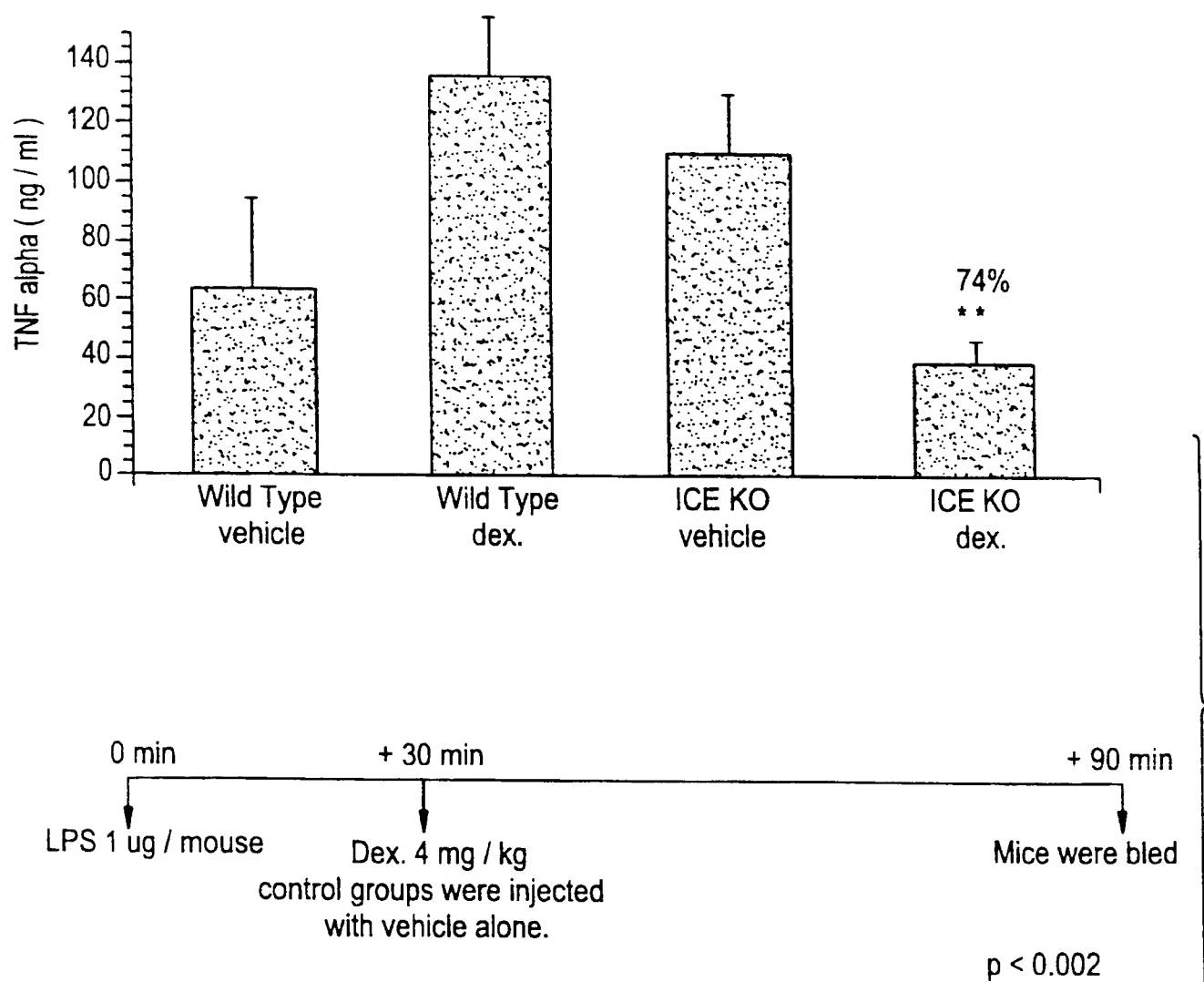
74. The method of claim 73, wherein the subject is resistant to a corticosteroid prior to administration of the agent.

75. The method of claim 73, wherein the subject is responsive to a corticosteroid prior to administration of the agent but exhibits increased sensitivity to the corticosteroid after administration of the agent.
- 5
76. The method of claim 73, wherein treatment of the subject with a corticosteroid is to be stopped and administration of the agent ameliorates a steroid rebound effect in the subject.
- 10
77. The method of claim 73, wherein the agent is an IL-18 antagonist, the agent being administered at a dosage and by a route sufficient to inhibit IL-18 activity in the subject.
78. The method of claim 73, wherein the agent is an Interleukin-12 (IL-12) antagonist, the agent being administered at a dosage and by a route sufficient to inhibit IL-12 activity
- 15
- in the subject.
79. A pharmaceutical composition comprising an agent which antagonizes a target that regulates production of interferon- γ (IFN- γ) in the subject, a corticosteroid and a pharmaceutically acceptable carrier.
- 20
80. The pharmaceutical composition comprising an inhibitor of a caspase family protease, a corticosteroid and a pharmaceutically acceptable carrier.
81. The pharmaceutical composition of claim 80, wherein the inhibitor is an ICE
- 25
- inhibitor.
82. The pharmaceutical composition comprising an anti-IL-12 monoclonal antibody, a corticosteroid and a pharmaceutically acceptable carrier.
- 30
83. The pharmaceutical composition of claim 82, which is formulated for topical administration.
84. The pharmaceutical composition of claim 82, which is formulated for administration by inhalation.
- 35
85. The pharmaceutical composition comprising an NK cell antagonist, a corticosteroid and a pharmaceutically acceptable carrier.

86. The pharmaceutical composition of claim 85, wherein the NK cell antagonist is an anti-asialo-GM1 antibody or an NK1.1. antibody.

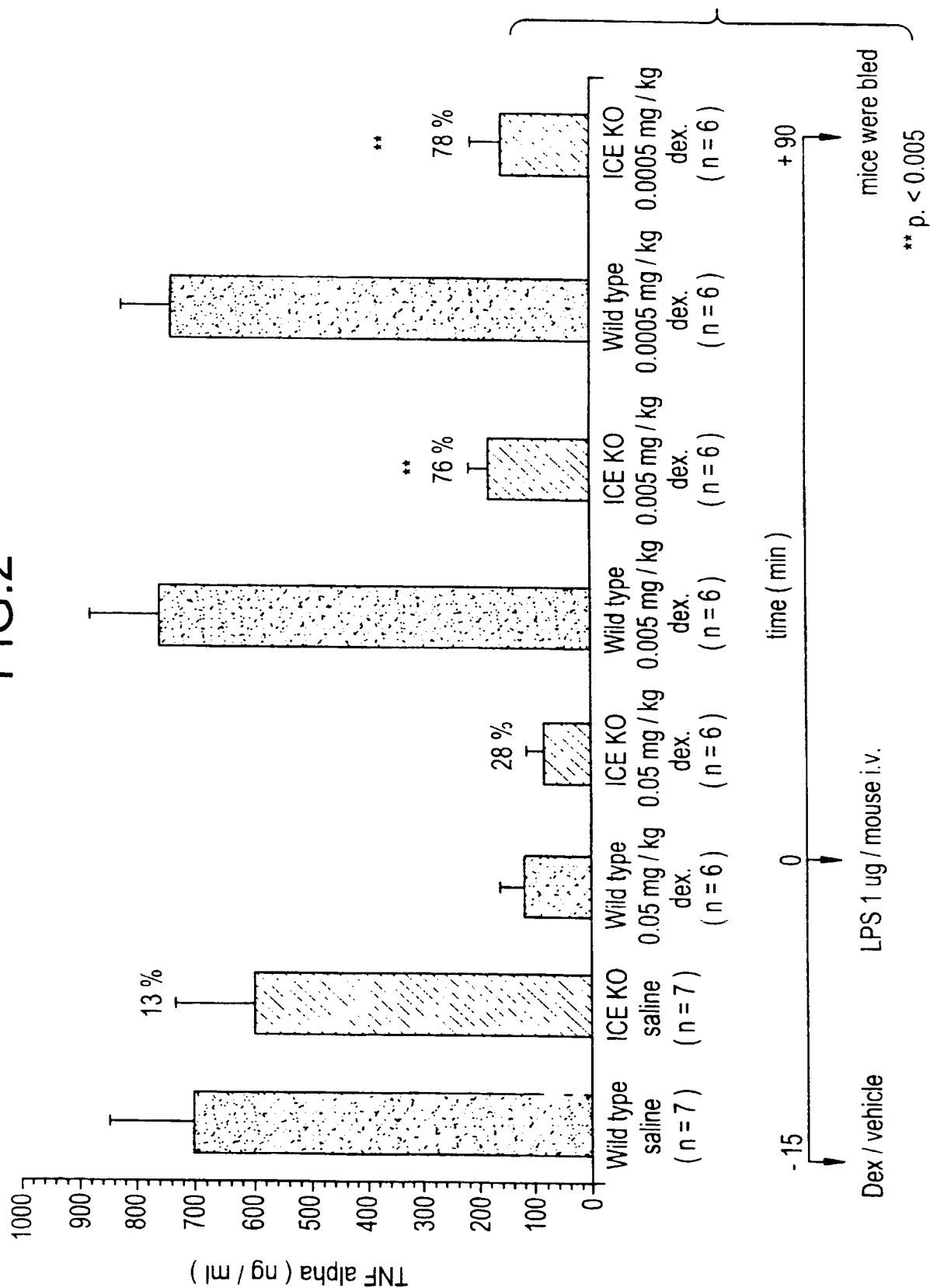
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FIG.1



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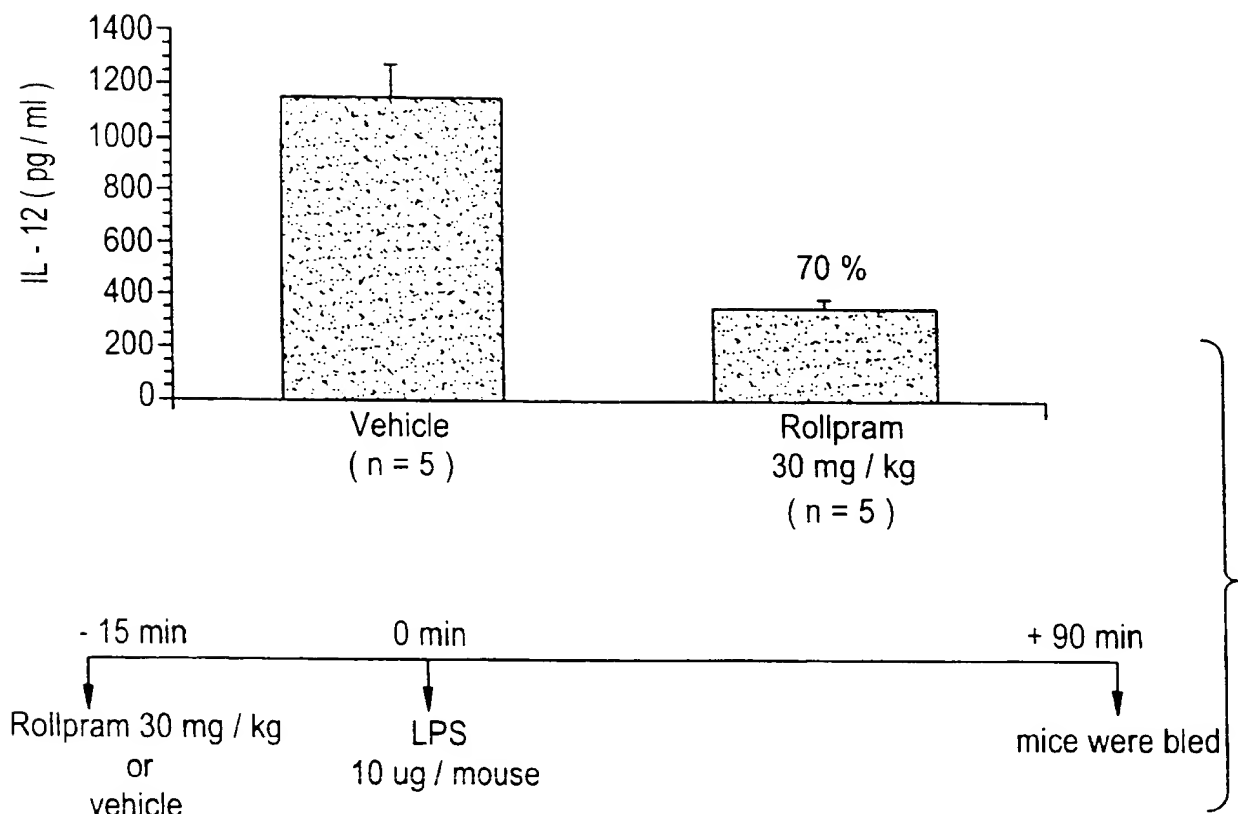
FIG.2



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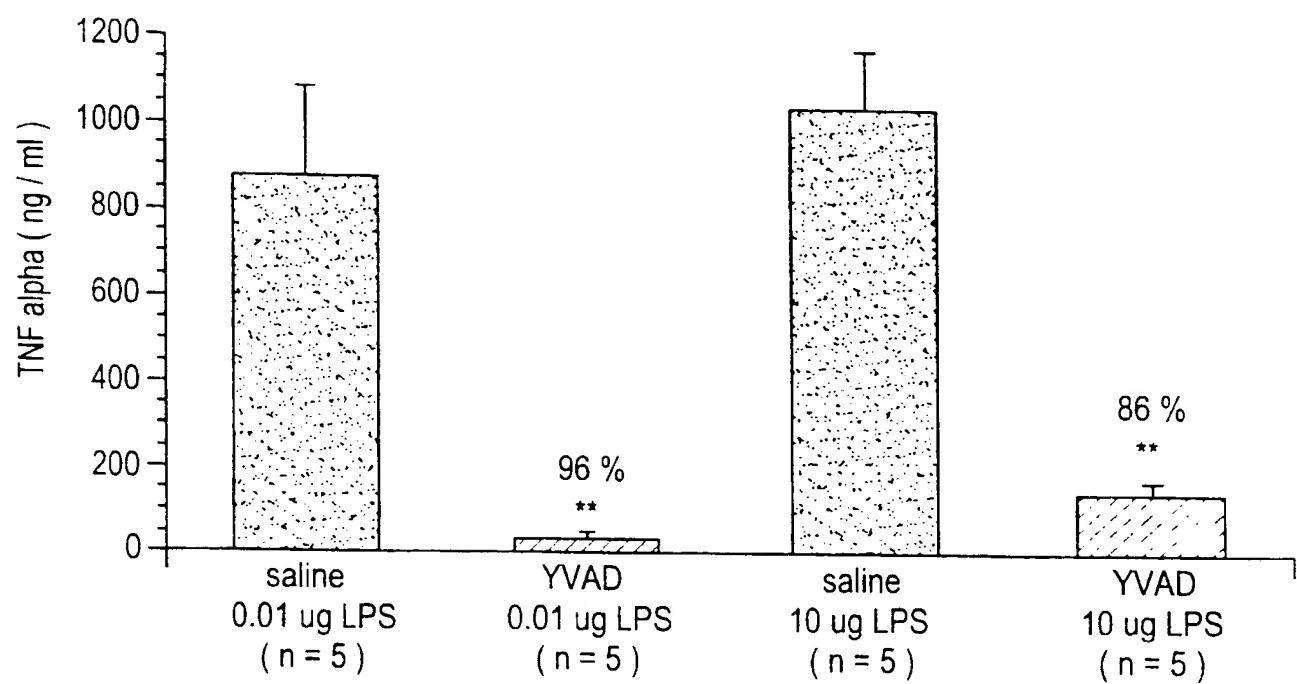
FIG.3



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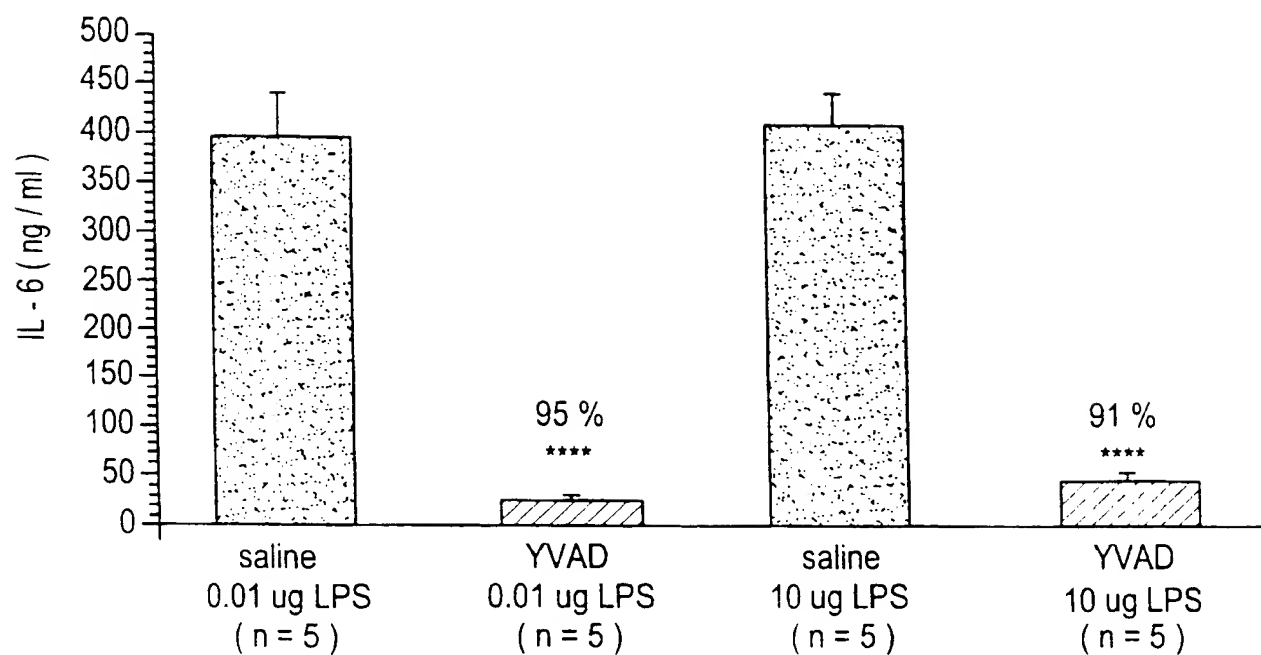
FIG.4



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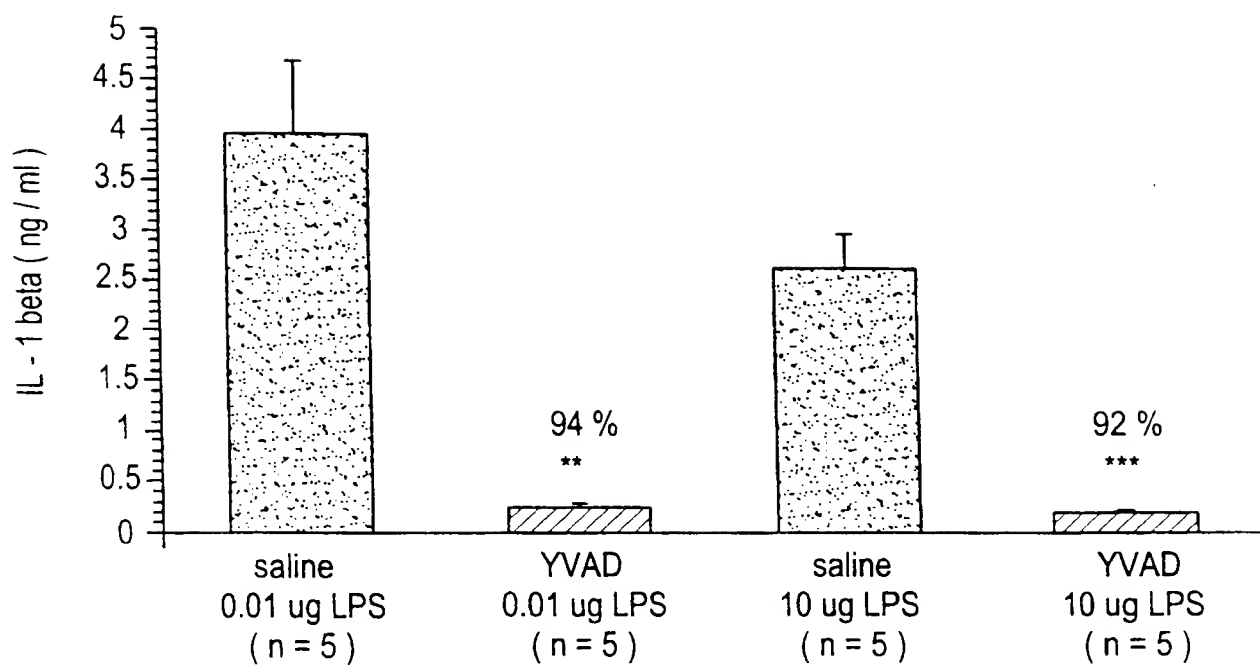
FIG.5



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FIG.6



SUBSTITUTE SHEET (RULE 26)



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 31/57	A3	(11) International Publication Number: WO 98/41232 (43) International Publication Date: 24 September 1998 (24.09.98)
(21) International Application Number: PCT/US98/04916 (22) International Filing Date: 12 March 1998 (12.03.98) (30) Priority Data: 08/820,692 18 March 1997 (18.03.97) US 09/016,346 30 January 1998 (30.01.98) US (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Applications US 08/820,692 (CIP) Filed on 18 March 1997 (18.03.97) US 09/016,346 (CIP) Filed on 30 January 1998 (30.01.98) (71) Applicant (for all designated States except US): BASF AKTIENGESELLSCHAFT [DE/DE]; D-67056 Ludwigshafen (DE). (72) Inventors; and (75) Inventors/Applicants (for US only): SEKUT, Les [US/US]; 4 Roy Street, Westborough, MA 01581 (US). CARTER, Adam [US/US]; 5 Pond Street, Newburyport, MA 01950 (US). GHAYUR, Tariq [PK/US]; 6 Oak Street, Grafton, MA 01519 (US). BANERJEE, Subhashis [IN/US]; 35 Hapgood		Way, Shrewsbury, MA 01545 (US). TRACEY, Daniel, E. [US/US]; 149 Shaker Road, Harvard, MA 01451 (US). (74) Agents: KARA, Catherine, J. et al.; Lahive & Cockfield, LLP, 28 State Street, Boston, MA 02109 (US). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> (88) Date of publication of the international search report: 5 October 2000 (05.10.00)
(54) Title: COMPOSITIONS FOR MODULATING RESPONSIVENESS TO CORTICOSTEROIDS (57) Abstract <p>Method for modulating responsiveness to corticosteroids in a subject are provided. In the method of the invention, an agent which antagonizes a target that regulates production of IFN-γ in the subject is administered to the subject in combination with a corticosteroid such that responsiveness of the subject to the corticosteroid is modulated as compared to when a corticosteroid alone is administered to the subject. In one embodiment, the agent is an IL-18 antagonist. In another embodiment, the agent is an interleukin-12 (IL-12) antagonist. In yet another embodiment, the agent is an NK cell antagonist. In a preferred embodiment, the agent is an inhibitor of a caspase family protease, preferably an ICE inhibitor. In another preferred embodiment, the agent is an anti-IL-12 monoclonal antibody. In yet another preferred embodiment, the agent is an anti-asialo-GM1 antibody or an NK1.1 antibody. Other preferred agents include phosphodiesterase IV inhibitors and beta-2 agonists. The methods of the invention can be used in the treatment of a variety of inflammatory and immunological diseases and disorders. Pharmaceutical compositions comprising an agent which antagonizes a target that regulates production of IFN-γ in a subject, a corticosteroid and a pharmaceutically acceptable carrier are also provided. A preferred composition comprises an ICE inhibitor, a corticosteroid and a pharmaceutically acceptable carrier.</p>		

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/04916

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/57

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94 03153 A (GLAXO GROUP LTD ; TAYLOR ANTHONY JAMES (GB); NEALE PHILIP JOHN (GB)) 17 February 1994 (1994-02-17) page 6, line 24 -page 7, line 29 ---	1,6,8, 11,12, 17,20,23
X	EP 0 416 950 A (GLAXO GROUP LTD) 13 March 1991 (1991-03-13) abstract ---	1,6,8, 11,12, 17,20,23
X	WO 96 32345 A (ASHURST IAN C ; LI LI (US); HERMAN CRAIG S (US); RIEBE MICHAEL T (U)) 17 October 1996 (1996-10-17) page 4, line 9 -page 5, line 4 ---	1,6,8, 11,12, 20,23
- / - -		

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

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"O" document referring to an oral disclosure, use, exhibition or other means

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Date of the actual completion of the international search

13 July 1998

Date of mailing of the international search report

23.07.98

Name and mailing address of the ISA

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Authorized officer

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/04916

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 19968 A (GLAXO GROUP LTD ;GREEN ALEXANDER PETER (GB)) 4 July 1996 (1996-07-04) page 3, line 24 -page 4, line 10 ---	1,6,8, 11,12, 20,23
X	WO 93 15741 A (GLAXO GROUP LTD) 19 August 1993 (1993-08-19) page 3, line 34 -page 4, line 10 ---	1,6,8, 11,12, 20,23
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X	US 3 320 125 A (MERCK & CO INC) 16 May 1967 (1967-05-16) claim 1 -----	1,6,8, 11,12, 17,20,23

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/04916

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 1-78 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.: 1-9, 11-86
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA 210

Continuation of Box I.2

Claims Nos.: 1-9,11-86

In view of the very large number of compounds which are defined by the wording of the claims and the fact that compounds cannot be sufficiently characterized by their pharmacological profile or their mechanism of action (agent which antagonizes a target that regulates ... , etc.), the search has been performed on the general idea and the combinations explicitly mentioned in the examples

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/04916

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